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EXPERIMENTAL DURUM MILLING AND MACARONI MAKING TECHNIQUE [1]

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Introduction

The production of durum wheat in western Canada has increased to a marked extent during the past twelve years. The principal areas devoted to the cultivation of this crop are the south and south-eastern portions of the provinces of Manitoba and Saskatchewan. Due to drought and rust conditions, durum wheat has constituted 30% to 55% of the total wheat acreage sown in the former province since 1928. Although Canada possesses a number of laboratories for the investigation of bread wheats, there were until recently no facilities available for making quality studies upon durum wheat. The present and prospective production of this wheat indicated the desirability of establishing a laboratory, which was recently undertaken by the National Research Council of Canada, after a survey by the junior author of the existing laboratory facilities of certain of the larger commercial organizations in the United States. This laboratory is at present located in the Dominion Grain Research Laboratory of the Board of Grain Commissioners at Winnipeg.

Obviously, one of the first requirements in such a program of investigation is the development of a standardized milling and processing technique, together with a quantitative definition of the various factors that, taken together, constitute what is generally known as "macaroni quality." The initial development of such standardized procedures, and of the necessary apparatus and equipment required to obtain these results has therefore been the main consideration of the investigations so far undertaken.

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In the initial stages of this work the authors received a great deal of valuable assistance and advice from various individuals connected with the laboratories previously mentioned, and their grateful thanks are due to all those who assisted in the establishment of this laboratory. This assistance was exceedingly valuable because of the fact that practically no information was available in the literature at that time regarding the experimental processing of macaroni.

The paucity of published information in this field indicates the desirability of some standard system of nomenclature. Until recently, macaroni products in general have been grouped together under the designation of "alimentary pastes," employing the names: macaroni, spaghetti, vermicelli, *etc.*, to describe certain specific shapes and sizes. In this work, however, we have adopted the suggestion of LeClerc (1933) to employ the term "macaroni" or "macaroni products," as a general designation. When a specific physical form is referred to it is indicated by the conventional name. A very comprehensive description of the various commercial types is given in LeClerc's paper.

Milling and Processing Equipment and Apparatus

Milling

The equipment for milling durum wheats consists of a two-stand Allis-Chalmers experimental mill and a small scale purifier. The mill is a standard unit with one bolter. Two stands of break rolls are employed, the first being equipped with two No. 14 and the other with two No. 20 Gantz cut rolls (6″ × 6″), both sets with 3/8″ spiral and running dull to dull. The purifier was especially constructed by the Strong Scott Company and is illustrated in Figure 1. It is fitted with four classifying sieves 9 inches wide as follows: head, 50 G.G., 40 G.G., 34 G.G., and 28 G.G. tail and is driven by an independent 1/2 h.p. motor. The dust collector was added at a later date and consists of a simple frame 18 inches × 18 inches × 18 inches, the sides and top being equipped with panels of 72 G.G. cloth and a drawer at the bottom for the removal of accumulated products.

Macaroni Processing Equipment

This consists of a small-scale mixer, kneader, and press mounted together as a unit and is identical in construction with that described by Fifield (1934). Certain additions, however, have been found necessary. These consist of (1) provision of limit switches to stop the operation of the motor at either end of the press travel to protect it against damage, and (2) the use of oil instead of water in the press jacket and the provision of adequate stirring and temperature control. Heating is effected by means of a 300 watt Nichrome resistor encased

in a pyrex glass tube, which was bent to a shape suited to the rather limited space available. Control is effected with a simple mercury thermoregulator and a relay. Stirring is essential and is obtained with a small motor-driven propellor permanently mounted on the jacket cover and controlled by a rheostat. The experimental unit with these additions is illustrated in Figure 2. Dies are available

Figure 1. Experimental purifier.

for the production of standard size macaroni and spaghetti and also a special die to be described later for the production of a flat strip.

Drying Equipment

This consists of two units, (1) a preliminary drying or fanning cabinet, and (2) a final drying cabinet. The preliminary drying cabinet is modelled upon the lines of the smaller one described by

Fifield (1934) but is fitted with a bimetallic (De Khotinsky) thermoregulator. The final drying cabinet has been described in detail by Binnington and Geddes (1934) and is illustrated in Figure 3. The air employed in drying is continuously re-circulated and conditioned, the apparatus functioning independently of variations in room temperature or humidity. Removal of moisture from the circulating air is

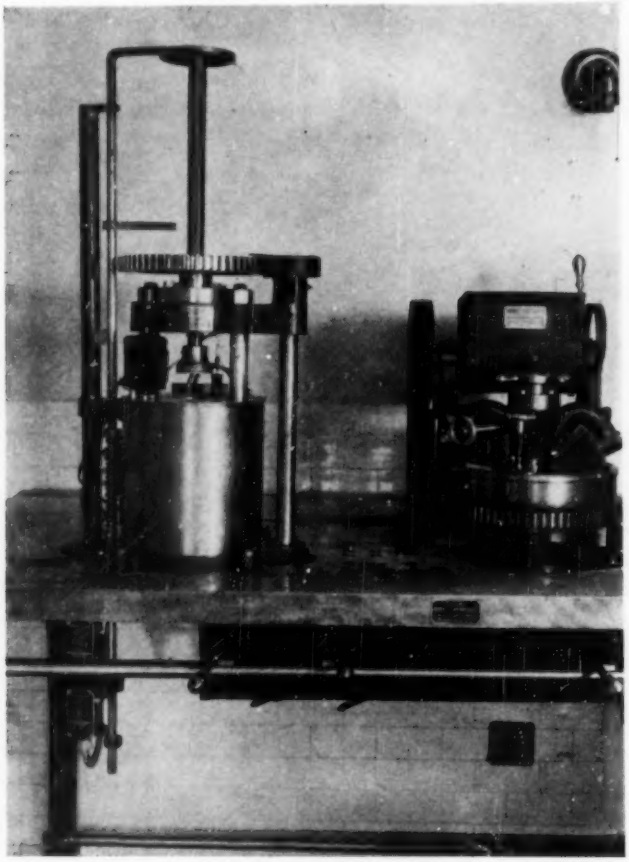

Figure 2. Experimental mixing, kneading, and pressing unit showing limit switches and temperature control equipment for press.

effected by condensation or freezing out upon cooling coils connected to an electric refrigerator unit, the temperature being restored and maintained constant by heating coils and a thermoregulator. Control of the rate of de-humidification is effected by by-passing an increasing volume of the circulating air over the cooling coils through the agency of a ratchet operated valve actuated from an electric clock fitted with contacts at 2-1/2 minute intervals and provided with independent

switches. By suitable adjustment of these switches the by-pass valve may be completely opened in 5, 10, 20, 30, 40, 60, or 120 hours. Precise control of the time-humidity relation is secured by a controlled steam input from a small electric (direct water resistance) boiler. The method of obtaining this control is similar to that described by Warren (1933) for producing a controlled temperature program in an electric muffle furnace. The desired humidity curve is first plotted and relative humidities read off for each hourly point. These are converted into wet bulb equivalents for the selected dry bulb temperature, plotted on a wet and dry bulb recording thermometer chart (or charts) and the points connected with a line. A disc of celluloid .065 inch thick is superimposed upon the chart and the two fastened together upon a

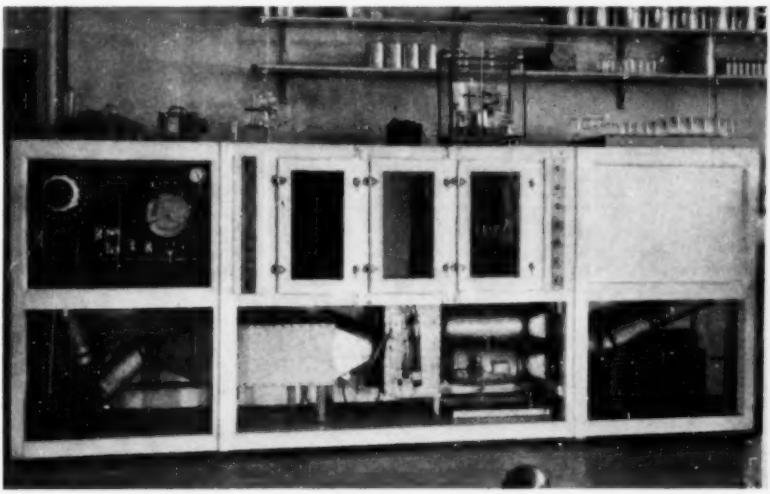

Figure 3. Experimental drying cabinet with automatic recording balance in position.

circular brass disc by means of a central bolt. A 1/2 inch wide strip of a mixture of precipitated copper powder and a cellulose lacquer is then painted on the celluloid in such a manner that the outer edge accurately follows the plotted line. After thorough drying, this painted strip is burnished with a smooth metal or glass object, a connecting wire attached, and copper plated on from a cyanide bath. After plating, the surface is lightly polished, and the finished control chart mounted on the recorder, which is fitted with an insulated brush contact bearing on the copper strip; the wet bulb pen serves as the other contact. In operation, a rheostat is connected in series with the direct resistance boiler and adjusted so as to maintain the water temperature just below the boiling point. The relative humidity of the circulating air within the cabinet is raised to 95%–100% and the control started.

As soon as the humidity falls to the predetermined starting point, the wet bulb pen contacts with the edge of the conducting strip, the resistance is shorted out of the circuit and steam is generated instantaneously and the humidity rises. The pen then draws away from the contact edge, throwing the resistance into the circuit again. This system of control (adding and withdrawing moisture simultaneously) is analogous to that employed in maintaining a thermostat below room temperature by the combined use of heating and cooling devices.

Milling Technique

Milling tests are conducted upon cleaned, weighed samples equivalent to 2500 grams at 13.5% moisture. These samples are conditioned to 13.5% moisture 24 hours before milling and tempered by raising the moisture content to 15.5% one to two hours prior to milling. Scouring may be performed before adding this last 2% of water, but preferably just prior to milling, which is conducted according to the flow sheet shown in Figure 4. Five breaks are employed, and as

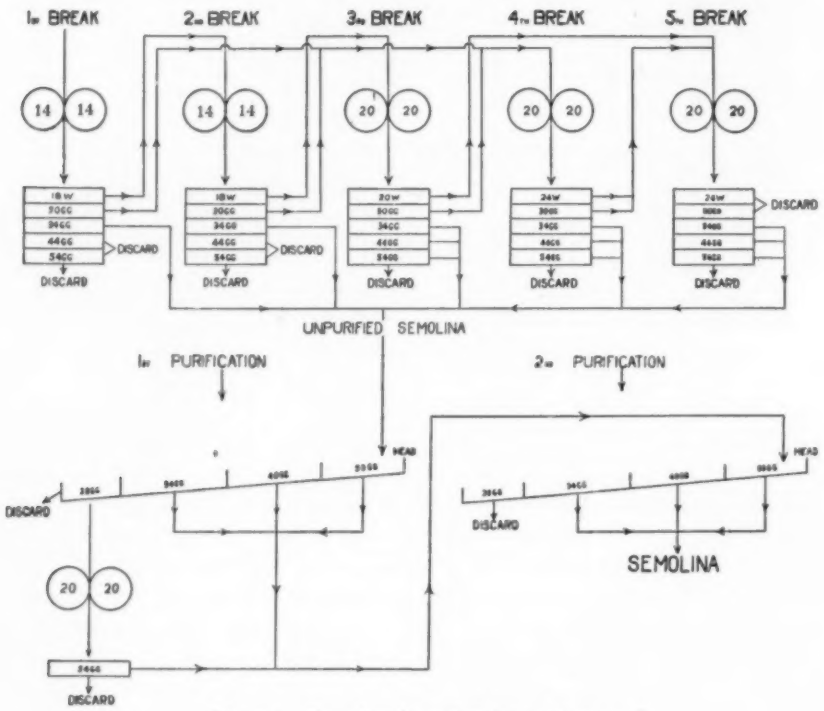

Figure 4. Flow sheet for milling durum wheats.

indicated in the flow sheet, the unpurified semolina includes all the material passing through a No. 30 G.G. sieve, and retained on a No.

54 G.G. sieve; this is all bulked together and purified, using a relatively small flow of air. The throughs from the No. 28 G.G. are reground, bolted on a No. 54 G.G., mixed with the throughs of the No. 50, 40, and No. 34 G.G. from the first purification, and re-purified using the maximum air flow attainable. The throughs from the No. 50, 40, and 34 resulting from this second purification are thoroughly mixed and represent the finished semolina.

Initially a 3-day tempering period was employed but considerable variation was found to exist in the carotene content of the semolina from replicate millings which was found to be associated with the presence of varying quantities of germ. Reduction of the conditioning period to one day appeared to minimize this variability. This is shown by the statistical constants, given in Table I, for carotene data

TABLE I

CAROTENE CONTENT OF SEMOLINA FOR TWO SERIES OF REPLICATE MILLINGS EMPLOYING DIFFERENT CONDITIONING PERIODS

Sample	Conditioning period	Number of replicates	Mean	Range	Standard deviation	Coefficient of variability
	days		*p.p.m.*	*p.p.m.*	*p.p.m.*	%
Average No. 1 C.W., 1934 Crop	3	20	3.06	2.52–3.60	.340	11.1
Average No. 1 C.W., 1933 Crop	1	30	2.67	2.53–2.78	.082	3.8

upon two series of replicate millings employing a 3-day and one-day conditioning period respectively. The material employed in the 3-day conditioning series consisted of an average sample of No. 1 C.W. Amber Durum wheat of the 1934 crop, but, unfortunately, after running 20 replicate millings, insufficient sample remained to repeat the series with a one-day conditioning period; accordingly, for the latter, 30 replicate millings were made of a similar sample but of the 1933 crop. Carotene content of the purified semolina was determined spectrophotometrically by the method of Ferrari (1933), using naphtha-alcohol solvent of the characteristics described by Geddes *et al.* (1934). The semolina samples were prepared for extraction by grinding in a Wiley mill fitted with a screen perforated with round holes 1/2 mm. in diameter.

Processing Technique

The experimental processing of macaroni products follows closely along commercial lines. The major operations in sequence are: mixing, kneading, pressing, fanning, and final drying or, as it is often termed, "curing." In addition to the possible effect on the finished

product of variations in these operations, absorption and duration of the rest period between kneading and pressing are also factors to be considered.

Before commencing an investigation of the possible effect of variations in processing treatment, it is necessary to attempt to obtain some quantitative measure of quality variations in the finished product. The characteristics of a good macaroni as defined by Le-Clerc (1933) are: hardness, brittleness, translucency, elasticity, and a rich amber colour. The fracture should be glassy, and long pieces should be sufficiently pliable to allow of considerable bending before breakage. In addition, a most important consideration is its behaviour on cooking. When boiled with water for ten minutes, a good macaroni will swell to at least twice its original size, will retain its tubular shape and firmness, will not become pasty, and will possess an agreeable odour. These qualities may be conveniently classified into three main groups: (1) colour, (2) physical properties other than colour, such as mechanical strength, translucency, vitreousness, *etc.*, and (3) resistance to disintegration upon boiling with water.

Preliminary experiments indicated that the colour of the finished product was quite susceptible to variations in processing prior to drying, whereas the other physical properties were conditioned largely by variations in the drying technique. Colour was therefore selected as a basis for evaluating the effects of variations in the processing treatment. The various quantitative methods of measuring and expressing colour are well known, and it is not intended to enter into an extended discussion of these methods here. Briefly, however, methods of colour measurement may be divided into two main groups: (1) methods that measure the *sensation* evoked by the colour stimulus and grouped together under the heading of "Colorimetry," and (2) methods that measure the *stimulus* itself. These methods are classed under the heading of "Spectrophotometry." Colorimetric methods involve the actual matching of colour, and therefore necessitate the use of colour standards and imply reasonably normal colour vision on the part of the observer. The results obtained by such methods, however, can be readily interpreted in terms of visual color. Spectrophotometric methods, on the other hand, involve no matching of color, but only the balancing of intensities at equal wavelengths. This feature also eliminates abnormalities of colour vision on the part of the observer. The results of such analyses are plotted in the form of spectral distribution curves, but unfortunately these cannot be readily interpreted as visual colour. Methods have been developed, however, for the conversion of such data into other systems of colour notation, although the calculations involved are rather complicated

and decidedly laborious. In view of the fact that the spectrophotometric method is fundamentally the basic one, and also that suitable equipment was available, it was selected for these studies. At a later date it is hoped to examine both methods critically with a view to selecting the one most suited to routine investigations. Preliminary studies indicated that none of the standard shapes of macaroni products was well adapted to the spectrophotometric method of colour analysis, and a special die was therefore secured enabling the production of a flat strip or ribbon, approximately 35 mm. wide by 1 mm. thick when dry, two such strips being extruded at the same time. For the purpose of colour analysis, discs are cut out of the undried strip with a large cork borer, 36 mm. internal diameter, and dried together with the remainder of the batch, by spreading upon sheets of bolting cloth stretched over wooden frames.

The instrument employed in these studies is a Bausch and Lomb universal spectrophotometer equipped with a suitable housing for reflection readings. Two 500-watt monoplane-filament stereopticon bulbs are used as illuminants, and are connected to a water-cooled rheostat. This enables a reduction of the filament voltage when taking readings in the brighter regions of the spectrum, and materially prolongs the life of the bulbs. The sample under test is fitted into a specimen holder which also carries a block of pure magnesium carbonate. Five settings of the instrument are made at a given wavelength, the relative positions of the standard white and the sample reversed, and five more readings taken. The average of these ten readings is then taken as the percentage reflection of the sample at the specified wavelength. Readings are taken at the following points through the visible spectrum (millimicrons): 700, 650, 600, 560, 540, 520, 500, 480, 460, and 440 (lower limit of visual perception). The average values obtained are plotted upon special graph paper (K. & E. No. N-355-60 R) and extrapolated to 400 millimicrons. Values are then read off and tabulated for each 10 millimicron point from 700 mμ. to 400 mμ. The results so obtained are calculated into terms of percentage Brightness, percentage Purity, and Dominant Wavelength.[4]

A preliminary comparison of the visual appearance of macaroni with the results of spectrophotometric analysis indicated that the factors associated with a desirable rich yellow colour were:

(*a*) Dominant wavelength in the region of 580 mμ.
(*b*) Relatively *high* purity
(*c*) Comparatively *low* relative brightness.

[4] Precise definition of these terms, together with details of the calculations involved, may be found in the "Colorimetry report, 1920–21," J. Optical Society of America, VI, Aug. 1922, and the "Spectrophotometry report, 1922–23," **10**: 169–241. A special slide rule designed to facilitate these calculations is manufactured by Keuffel and Esser, New York.

By the use of such experimentally obtained values, it should be possible to select the optimum conditions for each stage of processing. Obviously, however, the validity of the method is dependent upon the degree of uniformity that can be secured within a series of replicates. A visual examination of the freshly pressed macaroni indicated that the colour of the first portions extruded was appreciably lighter than that of the remainder. As 5 lots of strip approximately 4′ long were secured from a 600-gram batch, the first strips were discarded and discs cut and analyzed from the remaining four. For this series, the experimental material consisted of a large sample of No. 2 commercial semolina from which 8 replicate batches were processed according to the following technique: absorption, 30.8%; mixing period, 4 minutes; kneading period, 5 minutes; rest period in the press, 10 minutes; and press temperature, 90° F. After pressing, the material was allowed to air-dry until curling commenced and transferred to the drying cabinet. Drying was conducted at 90° F. under a falling humidity gradient for a period of 60 hours, the initial humidity being approximately 100% and the final value 40%. The above experimental conditions were based on the results of prior experiments in which colour and other properties were judged by simple inspection.

The labour involved in reading and calculating the results for four discs from each batch is very considerable, and for this reason the results obtained with discs 2 and 3 were compared with those for all discs, to ascertain whether the reading of only two discs might be utilized to secure reliable data on the colour of the sample. The batch means for 2 and 4 discs, respectively, are given in Table II. The

TABLE II

COLOR CHARACTERISTICS FOR REPLICATE PROCESSINGS

Batch number	Means for 2 and 4 discs per batch					
	Brightness		Purity		Dominant wavelength	
	2 Discs	4 Discs	2 Discs	4 Discs	2 Discs	4 Discs
	%	%	%	%	mμ	mμ
29	46.4	47.3	19.2	19.7	582.0	581.7
30	48.1	47.5	19.0	18.6	582.6	582.4
31	45.2	45.3	18.0	19.0	582.1	582.3
32	45.1	44.0	19.3	21.4	583.4	582.5
33	44.3	44.2	19.7	19.9	581.6	582.1
34	46.4	46.8	18.5	19.6	582.2	581.8
35	45.6	45.3	19.2	19.5	582.4	582.4
36	44.4	44.2	20.0	19.6	582.8	583.3
All batches	45.68	45.57	19.11	19.68	582.39	582.20

individual values were submitted to an analysis of variance, the total variance being divided into two components: (1) that due to the differences between batches and (2), that due to the differences within batches. The "F" values for the ratios between these two variances are given with their 5% points in Table III, together with the standard deviations and coefficients of variability, computed from the variances for "totals," "between batches," and "within batches."

Considering first the total variability, it will be noted from the coefficients of variability that purity is relatively the most and domi-

TABLE III

STATISTICAL CONSTANTS FOR BRIGHTNESS, PURITY, AND DOMINANT WAVELENGTH EMPLOYING 2 AND 4 DISCS RESPECTIVELY

F Values for $\frac{\text{Variance between batches}}{\text{Variance within batches}}$

	2 Discs		4 Discs	
	F	5% Pt.	F	5% Pt.
Brightness	2.26	3.64	3.94	2.57
Purity	0.32	3.73	0.98	3.41
Dominant wavelength	1.58	3.64	0.67	3.41

Standard deviations

	Total		Between batches		Within batches	
	2 Discs	4 Discs	2 Discs	4 Discs	2 Discs	4 Discs
Brightness	1.50	1.87	1.26	1.44	1.19	1.45
Purity	1.27	1.66	0.60	0.82	1.53	1.67
Dominant wavelength	0.73	0.71	0.58	0.30	0.65	0.73

Coefficients of variability

	Total		Between batches		Within batches	
	2 Discs	4 Discs	2 Discs	4 Discs	2 Discs	4 Discs
Brightness	3.3	4.1	2.8	3.2	2.6	3.2
Purity	6.6	8.5	3.1	4.2	8.0	8.5
Dominant wavelength	0.1	0.1	0.1	0.05	0.1	0.1

nant wavelength the least variable; moreover, the reading of two discs only from each batch gives approximately similar values to those for 4 discs. The "F" values for the ratio of the variance "between batches" to that for "within batches" only exceed their corresponding 5% points in the instance of brightness for 4 discs, indicating, with this single exception, that the differences between the means for batches recorded in Table II are not significant. The means for brightness, purity, and dominant wavelength are in fairly close agreement whether determined on the basis of 2 or 4 discs and the results

on this series indicate that a fairly satisfactory measure of the variability and mean values for replicate tests may be secured by reading 2 discs, thereby greatly reducing the labour involved. Since the standard error of the mean of two determinations equals the standard deviation $\times \sqrt{2}$, the standard error of the difference between the means of two sets of duplicate determinations equals the standard deviation $\left(\frac{\text{S.D.} \times \sqrt{2}}{\sqrt{2}}\right)$. Taking the level of significance as twice the standard error, a difference between the mean values of any two semolinas for brightness, purity, and dominant wavelength would have to equal or exceed 3.00, 2.54, and 1.46 units respectively, in order to be significant, on the assumption that the variations within other samples would be of the same order of magnitude as those obtained in this series of replicates.

Having thus secured a measure of the differences required for significance in each of the colour characteristics, studies were then undertaken on the effect of varying the processing conditions and also to determine whether variations between replicate millings must be considered in evaluating durum wheat quality.

These conditions, taken in logical order, are: absorption, mixing, kneading, duration of rest period between kneading and pressing, fanning, and final drying or curing. The material employed in these studies consisted of a large sample of No. 2 commercial semolina, and the processing conditions (with the exception of the variable under investigation) were identical with those previously outlined, namely: 4 minutes mixing, 5 minutes kneading, 10 minutes rest period in press at 90° F., press temperature 90° F., no fanning, and drying at 90° F. for 60 hours under a falling humidity gradient of 100% to 40% relative humidity. This gradient is shown graphically in Figure 6, and will be discussed under drying studies.

Absorption

A series of batches was processed, in which the absorption was varied from 27 to 32% in increments of 1%, expressed on a 13.5% moisture basis. Absorptions of 27 and 28% produced a tight mix, with a definite tendency towards curling at the edges of the freshly extruded strip, 29 and 30% appeared to be quite satisfactory, while 31 and 32% produced a slack mix. Results of the colour analyses are given in Table IV and presented graphically in Figure 5. It will be noted that increasing absorption results in a definite and continuous *decrease* of brightness, and a similar but definite *increase* in purity, while dominant wavelength remained practically unaltered.

TABLE IV

EFFECT OF VARIATIONS IN PROCESSING TREATMENT UPON COLOR CHARACTERISTICS

Treatment	Brightness	Purity	Dominant wavelength
	%	%	mμ
Absorption 27%	51.5	17.0	580.0
Absorption 28%	48.6	19.0	580.0
Absorption 29%	43.2	22.5	581.0
Absorption 30%	41.1	21.0	581.0
Absorption 31%	39.5	22.0	581.0
Absorption 32%	37.1	24.0	582.0
Kneading time 2 mins.	46.4	20.1	582.7
Kneading time 4 mins.	49.1	18.9	581.9
Kneading time 5 mins.	42.2	22.1	581.0
Kneading time 8 mins.	45.1	18.9	585.6
Kneading time 12 mins.	47.1	19.5	576.0
Kneading time 15 mins.	45.1	17.1	582.5
Rest period 0 mins.	46.9	17.5	581.5
Rest period 5 mins.	45.2	19.9	581.2
Rest period 10 mins.	42.2	22.1	581.0
Rest period 15 mins.	45.1	19.9	581.3
Rest period 20 mins.	46.9	18.9	581.9

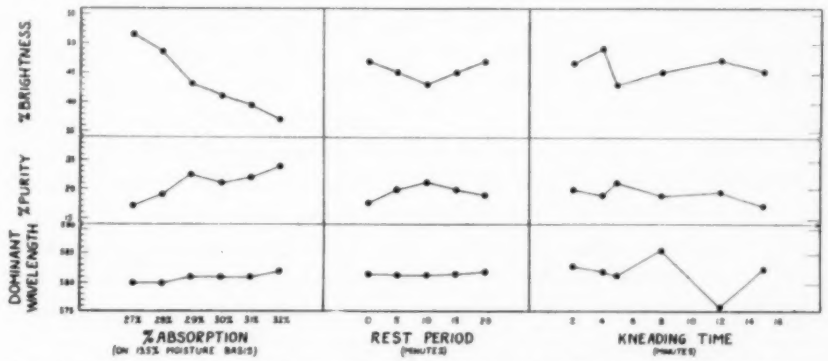

Figure 5. Effect of variations in absorption, rest period and kneading time upon percentage brightness, percentage purity and dominant wavelength of macaroni.

Considering these values in the light of the statistical criteria previously established, it will be seen that a change of 1% in absorption produces in general no significant difference in brightness, purity, and dominant wavelength. It is apparently necessary to alter the absorption at least 1.5% before a significant change can be noted in brightness and purity and 5% in the case of dominant wavelength. The optimum absorption for this particular sample of semolina would thus appear to fall in the range of 29–30%, and coincides with the optimum physical characteristics of the dough. In the later studies to be described employing this same semolina, 29.5% absorption was accordingly adopted.

Mixing

This operation is conducted by introducing 600 grams semolina (13.5% moisture basis) into the mixer which is then started and the calculated amount of water gradually added, distributing it well over the entire surface of the semolina. Mixing is continued until the mass assumes a uniformly granular appearance. This occupies approximately four minutes. Qualitative tests indicated that no appreciable effect was produced by minor variations in this time and as no particular advantage is to be gained by prolonging the mixing period, four minutes was selected without further quantitative investigation of colour change.

Kneading

This process is carried out in a rotating pan, the dough being formed into an endless strip by means of a small plow. The actual kneading is performed by corrugated steel rollers that idle upon the surface of the dough and may be forced into it by means of a screw adjusting device. Qualitative tests having indicated that kneading time produced an appreciable effect upon colour, a series of samples was processed at 2, 4, 5, 8, 12, and 15 minutes respectively. No difficulty was experienced in handling the doughs produced over this time range. Colour analysis results are given in Table IV and presented graphically in Figure 5. Brightness rises slightly between 2 and 4 minutes, drops rapidly at 5 minutes and then increases slowly to 12 minutes. The differences between intervals are only significant in the case of the 4 to 5 minute change. The differences between purity for intervals are only significant from 4 to 5 and 5 to 8 minutes, maximum purity being obtained with 5 minutes kneading. Dominant wavelength shows significant and irregular variations for the 8–12 and 15 minute periods. The optimum kneading time would thus appear to be 5 minutes.

Duration of Rest Period

The desirability of a rest, or temperature conditioning period between kneading and pressing is particularly emphasized when employing the flat strip die. Unless the dough is brought up to the press temperature, a thermal, and therefore plasticity gradient is set up between the material adjacent to the cylinder walls and the center of the dough body. This results in a more rapid extrusion at the ends of the slots in the die and consequently a wrinkling or curling of the pressed strip. Difficulty is then experienced in securing satisfactory discs for colour analysis. In commercial practice, the rest period is frequently carried out in a heated room, but from the standpoint of experimental procedure, it is more satisfactory to rest within the press

itself. Accordingly, after the kneading operation is complete, the strip of dough is cut into short lengths, rolled and introduced into the press cylinder. The ram is then run down until the dough is under slight pressure.

As in the case of kneading, variations in the time of resting were found to give definite qualitative differences in macaroni colour, and the effect of these variations was therefore investigated, 0, 5, 10, 15, and 20 minute periods being selected. No serious alterations in dough characteristics were produced by any of these variations, although a steady increase in plasticity, together with a smoother surface, resulted with increasing time. The colour analysis results are given in Table IV and graphically presented in Figure 5. The differences between intervals are barely significant in the cases of percentage brightness and purity, and are insignificant throughout the whole range for dominant wavelength. The general trends, however, indicate a significant minimum of brightness at ten minutes and a maximum of purity at the same point. The optimum time of rest would thus appear to be ten minutes.

Pressing

In small experimental equipment of the type used in these studies, the press is mechanically driven by means of a screw and a train of gears. No variations can thus be introduced in either the pressure applied, or its rate of application. The only factor that can be altered is press-jacket temperature.

The original equipment as supplied, was fitted with a standard type of 500 watt immersion heater; no control was provided, and the capacity of the heating unit was considerably in excess of that required. It was, therefore, replaced by a 300 watt heater as previously described, controlled by a simple mercury thermoregulator and a suitable relay. Early tests made with the apparatus in this form indicated that some source of variation existed which was finally traced to wide temperature differences in various parts of the oil-jacket. A motor driven stirrer was, therefore, fitted and was found to completely eliminate these difficulties.

The selection of a suitable press temperature is conditioned mainly by the physical properties of the freshly pressed product, such as plasticity, curling at the edges and a tendency to stretch and drop off the supports during the early stages of drying. Under these circumstances, colour is not the controlling factor and a detailed quantitative investigation was not undertaken. In general, however, the effect of increasing press temperature upon colour has been found to be very similar to that of increased time of rest. From the standpoint of

physical properties a jacket temperature of 90° F. appears to be the optimum for flat strip, but for macaroni and spaghetti, temperatures up to 110° F. may be used.

Fanning

The fanning operation consists of a rapid surface drying of the freshly pressed material with air of low humidity and comparatively high temperature. It is employed in commercial practice to prevent elongation and stretching of the goods under their own weight.

From an experimental standpoint and particularly where the strands are shorter than the standard commercial lengths, its use would not appear to be essential. Where low atmospheric humidities are encountered, a simple air drying for 5 to 15 minutes appears to suffice. Employing a normal absorption, no difficulty has been experienced from elongation in our work and fanning has accordingly been eliminated.

Drying

The final drying or curing operation is generally considered to represent the most critical stage of the entire manufacturing process. Too rapid drying results in curling, cracking, and checking, and affects both the cooking and physical properties of the dried product. Too slow drying, on the other hand, induces biochemical changes and results in souring and the growth of molds. Satisfactory drying can only be obtained under conditions involving a gradual reduction in the relative humidity of the air circulating over the macaroni. The ideal drying condition would appear to be one in which the reduction of relative humidity parallels or very slightly exceeds the rate of diffusion of moisture within the sample being dried.

In the best forms of commercial practice, the falling humidity gradient is obtained by a gradual and progressive increase in temperature, accompanied by the venting of a certain amount of air. This involves the continuous alteration of two variables, and it seemed desirable from an experimental standpoint to eliminate one of these. Accordingly, the drying equipment previously described was designed so as to allow of controlling the time-humidity gradient, by lowering the humidity while maintaining the temperature at a constant value. Control by raising the temperature, however, can also be obtained with this apparatus.

In selecting the drying rate employed in these studies, we were guided by the data presented by Hoskins (1932) relating to the commercial drying of macaroni. The humidity-gradient and moisture-loss curves plotted from these data are illustrated in Figure 6. This gradient represents a fall of humidity from 93% to 68% during a

period of 48 hours. These values are shown in comparison with the experimental humidity curve employed in our studies and a typical moisture loss curve obtained by means of an automatic recording balance. It will be noted that the experimental humidity curve falls from 100% to 40% relative humidity during a period of 60 hours. The lower final humidity and longer time were adopted because of the very low (20–30%) atmospheric humidity prevailing in this region during the winter months. The final moisture content of this sample was 9.43%.

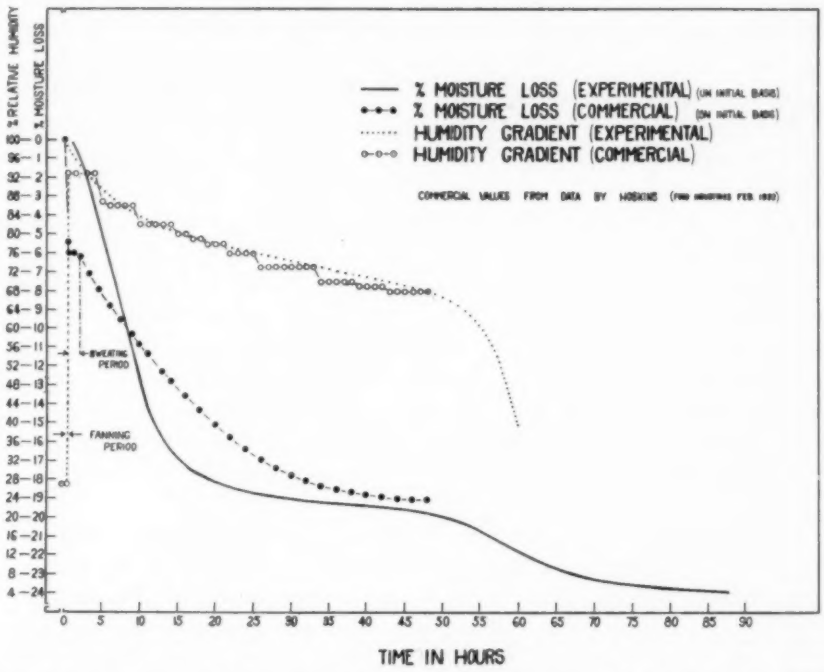

Figure 6. Commercial and experimental humidity gradients employed in drying macaroni with corresponding weight loss curves.

Examination of the data presented in Figure 6 shows that the commercial humidity gradient was produced in a series of steps, although the weight-loss data give a smooth curve. The experimental humidity gradient was calculated to follow the commercial as closely as possible, but in spite of this, certain differences in the moisture loss curves are apparent. Unfortunately, Hoskins does not give the final moisture content of his sample, and it is therefore difficult to draw a full and direct comparison between the two sets of results. It is very likely that his sample possessed a lower initial moisture content at the start of drying, as the moisture loss during mixing and kneading

would probably be greater with commercial than with experimental equipment, due to the longer times required for processing the larger masses of dough.

The establishment and interpretation of these moisture-loss and humidity curves have been greatly facilitated by the use of the automatic recording balance described by Binnington and Geddes (1936). In order to secure data for the interpretation of the drying curves obtained with this apparatus, a study was made of the equilibrium moisture content of macaroni when dried down to a series of decreasing humidity levels. The final value employed was 41.6% relative humidity and the vacuum-oven moisture of the sample at this point was 10.2%, and, calculated to an air-dry basis, 11.36%. The temperature employed in this study was 90° F. and a sample of experimentally milled semolina was used. The processing conditions were identical with those previously employed. The results obtained are illustrated graphically in Figure 7, together with a moisture regain curve plotted from data by Lindsay (Carrier Engineering Corp.) (1929). Time has not permitted a complete check of these latter data, but we have determined a number of points on the regain curve and have obtained excellent agreement with the above data. It will be noted that the falling humidity and regain values essentially coincide within the region of 40 to 80% relative humidity. Below and above these values the curves diverge, but, as macaroni is never dried in practice to below 40% relative humidity, the falling values are of greater significance from a practical standpoint than those obtained by the method of regain.

Utilizing the data presented in Figure 7 for equilibrium moisture values (descending humidity), and from a knowledge of the rate of moisture loss between definite increments of humidity, it is theoretically possible to construct an ideal moisture loss curve for any given drying time and, also, the time-humidity gradient necessary to obtain this moisture loss. Data are now being accumulated to this end, but the humidity gradient at present employed yields a product of satisfactory physical properties and provides a starting point for a more complete investigation of the whole problem of drying macaroni.

Tentative Experimental Macaroni Processing Technique

On the basis of the results obtained on the effects of variations within different stages of the processing technique, the following standardized procedure has been tentatively adopted.

600 g. of semolina, 13.5% moisture basis, is placed in the mixer, which is started, and the necessary amount of distilled water added gradually, distributing it well over the surface of the semolina. After

mixing for 2½ minutes, the mixer is stopped, and the dough cleared from the pins in the base of the mixing vessel. The operation is restarted and continued for another 1½ minutes. This clearing operation occupies about 1/2 minute, so that the entire mixing procedure is timed for a total of 4½ minutes.

The correct amount of water to add is best determined by a preliminary mix and knead, as the dough consistency cannot be judged until about 3 minutes of the mixing period has elapsed. At

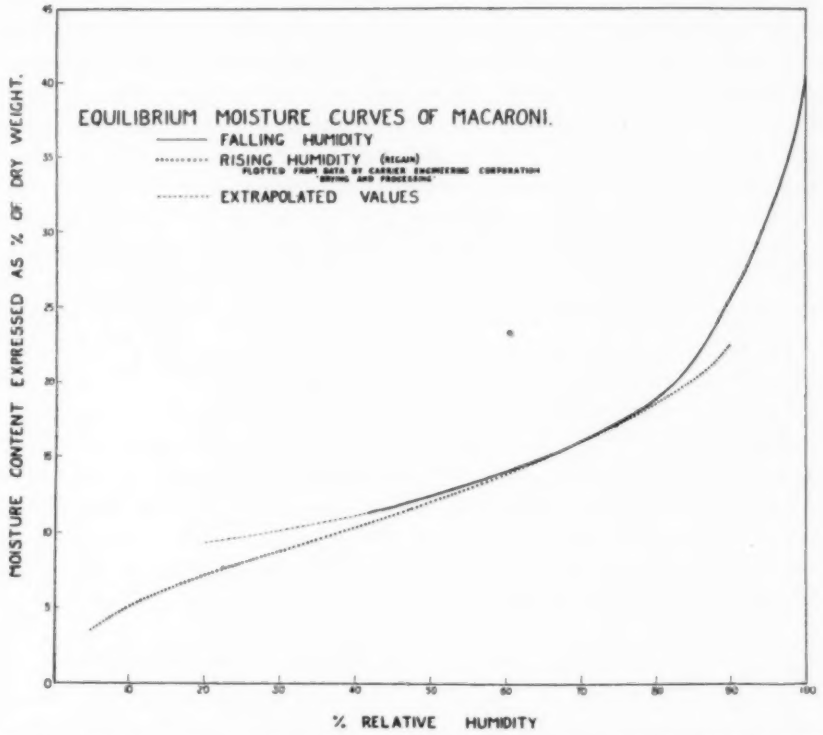
EQUILIBRIUM MOISTURE CURVES OF MACARONI.
FALLING HUMIDITY
RISING HUMIDITY (REGAIN)
PLOTTED FROM DATA OF CARRIER ENGINEERING CORPORATION
EXTRAPOLATED VALUES
MOISTURE CONTENT EXPRESSED AS % OF DRY WEIGHT.
% RELATIVE HUMIDITY

Figure 7. Moisture content of macaroni in equilibrium with air at various relative humidities, together with the hygroscopic regain curve of Lindsay (1929).

this time, if *insufficient* water has been employed, the dough will tend to form small masses, rather than a uniform granular body. Any insufficiency of water can be partly corrected at this point by suitable additions. On the other hand, if excess water has been added, its presence cannot be detected until during the kneading operation, at which stage no further adjustment can be made.

After the absorption has been determined and the mixing operation completed, the dough is transferred to the kneader and formed by hand into a continuous strip about 2½ inches wide extending around

the circumference of the pan. The plow is adjusted to bear upon the edge of this strip and the rolls brought into contact with its upper surface. Kneading is then commenced, and continued for a period of 5 minutes. During this time, the pressure of the rolls is gradually increased, and the plow moved outwards. Too rapid an application of the pressure will result in a tendency for the dough strip to lift, and the operation may be controlled by keeping the pressure slightly below this point. If the correct absorption has been employed, the dough will be smooth and uniform in appearance, but hard and stiff to the feel. It is then removed from the kneader by cutting the strip, lifting one end, and rotating the pan. The strip thus obtained, is cut into short lengths, rolled and packed into the press cylinder, the temperature of which has been previously adjusted to 90° F. ± 0.5°. The ram is then brought down until the dough just commences to issue from the die and the whole allowed to rest for ten minutes, the time being taken from the introduction of the sample. Pressing is then commenced, the extruded material being cut off in suitable lengths and folded over 5/8 inch wooden rods resting on suitable supports. When pressing is complete, the batch is allowed to stand until a tendency to curl is noticed (approximately 10 minutes at 30% humidity). It is then placed in the drying cabinet, which is maintained at 95% relative humidity. When the last batch has been placed in the cabinet, the humidity is raised to 98–100% and maintained at this level for about an hour, at which point the control chart is fitted and the drying operation commenced. At the expiration of the drying period, the heat and humidity controls are shut off, the fan only being kept running for a further period of 4–8 hours in order to allow the samples to attain room temperature gradually.

Macaroni Color Variability in Testing Durum Wheats

The color variability between and within replicate batches of macaroni processed from one sample of semolina has been dealt with in a previous section of this paper and employed as a basis for evaluating the significance of the effects of color variations due to modifications in processing technique. Assuming that the variations within the processing of other samples by the same procedure were of the same order as those for the sample investigated, it was pointed out that in processing only one batch and reading two discs the differences between the means of the duplicate values for brightness, purity, and dominant wavelength for two semolinas would have to equal or exceed 3.00, 2.54, and 1.46 units respectively, in order to be significant. In durum wheat testing, however, an additional variable is introduced by the experimental milling process and in order to secure a basis for

the evaluation of a series of durum wheat samples it is necessary to secure data regarding the total variability of the combined milling and processing operations.

With this end in view, 19 sub-samples of a uniform lot of an average No. 1 C.W. Amber Durum wheat grown in 1933 were milled and one batch of macaroni processed from each by the respective procedures already outlined. Two discs were taken from each batch for spectral analysis, but in this series the reflection data were averaged prior to calculating the brightness, purity, and dominant wavelength. In addition, carotene determinations were made in duplicate on each semolina. Yields and mean carotene values for the semolina, and color analysis data upon the macaroni are recorded in Table V and the statistical constants in Table VI.

TABLE V

YIELD, CAROTENE, AND MACARONI COLOR DATA FOR REPLICATE MILLINGS OF NO. 1 C.W. AMBER DURUM WHEAT

Replicate number	Semolina		Macaroni		
	Yield	Carotene	Brightness	Purity	Dominant wavelength
	%	*p.p.m.*	%	%	mμ
1	32.0	2.72	41.8	25.0	579.7
2	31.2	2.68	42.7	25.3	579.0
3	32.4	2.57	43.6	26.5	579.0
4	31.4	2.73	43.0	27.6	579.3
5	32.0	2.64	44.4	24.8	579.5
6	32.2	2.48	43.1	29.0	578.9
7	32.4	2.48	41.1	28.9	579.6
8	31.8	2.72	41.7	30.0	578.9
9	31.6	2.72	43.4	28.3	578.2
10	32.0	2.62	46.1	26.3	580.0
11	31.0	2.78	46.4	27.6	579.3
12	32.0	2.66	47.3	23.0	580.0
13	33.0	2.64	47.3	24.2	579.2
14	32.0	2.63	46.6	25.8	579.3
15	32.5	2.52	45.5	25.8	579.3
16	31.5	2.65	48.7	22.9	579.0
17	31.5	2.62	45.1	25.8	579.2
18	31.6	2.60	46.0	25.9	579.5
19	31.6	2.53	47.1	23.0	580.0

Considering first the variability in yield and carotene content of semolina, it will be noted that both are relatively low. The standard deviations for brightness, purity, and dominant wavelength may be compared with those for "between batches" in Table III for replicate processings of a single lot of semolina in which the individual data rather than the means were analyzed statistically. This comparison shows that the values involving both the milling and processing

TABLE VI

STATISTICAL CONSTANTS FOR YIELD, CAROTENE, AND MACARONI COLOR DATA FOR REPLICATE MILLINGS OF NO. 1 C.W. AMBER DURUM WHEAT

	Mean	Standard deviation	Coefficient of variability
Yield	31.88	.492	1.5
Carotene	2.631	.086	3.3
Brightness, %	44.78	2.222	5.0
Purity, %	26.09	2.081	8.0
Dominant wavelength	579.31	0.448	0.1

Simple correlation coefficients

	Yield	Carotene
Carotene	−.544	
Brightness, %	−.033	−.007
Purity, %	−.031	.021
Dominant wavelength	.112	−.021

At 5% point $r = .456$.

variables are greater than those for processing only. Assuming that the variances between duplicates in the series involving both variables is of the same order of magnitude as those determined for the processing variable only, the error variances, *i.e.* for "within batches" may be employed as a measure of the significance of the differences between replicates in the present series. Computations made on this basis, show that there is a significant difference between replicates in the instance of brightness, and purity, but not in the case of dominant wavelength. Differences in milling, therefore, appear to introduce variability into the evaluation of durum wheat quality from the standpoint of the purity and brightness values for the macaroni. However, in the present series, the 19 samples employed were processed on two separate days, whereas the previous series of 8 replicates were processed on the same day, and it is possible that a day-to-day variation has contributed to the variances attributed to milling.

The standard deviations recorded in Table VI serve as a basis for establishing criteria of the differences required between the several analytical values in order that two durum wheat samples may be clearly differentiated on the basis of a single milling and macaroni test and averaging the reading of two discs. Since the standard error of the difference between any two values equals the standard deviation $\times \sqrt{2}$ and the level of significance is twice the standard error, the differences required would be approximately three times the standard deviations recorded in Table VI. On this basis the differences between the values for semolina yield, carotene content of semolina and

brightness, purity and dominant wavelength would have to equal or exceed 1.5%, 0.26 p.p.m., 6.7%, 6.2%, and 1.4 millimicrons, respectively.

The simple correlations given in Table VI are all insignificant with the exception of that for semolina yield and carotene content. The explanation of this negative relationship appears to lie in the fact that a low semolina yield in this series is associated with an increase in the quantity of flour produced rather than with excessive purification. The production of larger quantities of flour implies finer grinding of germ rendering its removal from the semolina more difficult.

Discussion

In the work reported, the primary objective has been the development of equipment and standardized experimental technique for investigating durum wheat quality, and also to attempt a quantitative definition of some of the factors constituting macaroni quality. Thus far, only the milling and processing technique together with the color characteristics of the finished product have been studied in detail. Despite the use of a standardized technique throughout, the variability in brightness and purity of the finished product is somewhat high and it will probably be necessary in testing different durum wheats for these characteristics to employ a series of replicates to render the test sufficiently precise. However, the precision of the spectrophotometric procedure may be increased by the substitution of a photoelectric method in place of the visual one now employed, which will render it possible to secure spectral data at shorter wavelengths and thus avoid the necessity of extrapolating the spectral distribution curves to the limits of the visible spectrum. The visual method is also too laborious for large numbers of routine tests and the possibilities of the photoelectric and also the colorimetric method are accordingly being investigated.

Aside from color, the measurement of physical properties of the macaroni such as transverse and torsional breaking strength and also its cooking properties are under investigation, but sufficient data have not been accumulated to justify any statements as to their probable utility in distinguishing between the products made from wheats of different quality.

Summary

The small scale durum milling, processing, and drying equipment of the Durum Research Laboratory of the National Research Council of Canada is described. This comprises (*a*) a two-stand experimental mill with suitably cut rolls and a small purifier, (*b*) macaroni processing

equipment, consisting of mixer, kneader, and press mounted together as a unit, and (*c*) a drying cabinet, within which definite time-humidity drying gradients may be produced.

Standardization of the experimental milling technique has been carried out with particular reference to uniformity in the pigment content of the semolina and a milling procedure is described and a flow sheet presented. Employing the one-day conditioning period selected, the carotene content of the purified semolina varied over a range of 0.25 p.p.m., the standard deviation being 0.082 p.p.m., and the coefficient of variability 3.8%.

A spectrophotometric procedure for the measurement of macaroni color employing discs cut from a flat strip of macaroni produced by means of a special die is described, the results being expressed in terms of percentage Brightness, percentage Purity, and Dominant Wavelength, and the significance of these values in relation to the visual appearance discussed. The variation of these color characteristics within a series of replicate processings has been studied and employed as a basis for evaluating the effect of variations in processing technique.

Increased absorption resulted in a definite and continuous decrease of brightness and increase in purity, dominant wavelength remaining practically unaltered. Increased time of kneading produced an irregular effect upon brightness, the minimum brightness being obtained with a kneading time of 5 minutes. Purity and dominant wavelength also varied in an irregular manner, the maximum purity occurring at the 5 minute time.

Increasing the duration of the rest period in the press between kneading and pressing resulted in a decrease of brightness to a minimum at 10 minutes followed by a steady increase. With purity, the reverse condition was found, the maximum value occurring at 10 minutes. Dominant wavelength was practically unaltered.

The selection of a suitable press temperature was conditioned principally by the plasticity of the freshly pressed macaroni, and color was therefore not investigated exhaustively. In general, however, variations in this temperature produced similar results to those of altered time of rest. It was found essential to control the press temperature accurately.

The fanning operation employed in commercial practice for preliminary drying was eliminated by using a short air drying prior to the final drying or "curing" operation.

A tentative humidity gradient covering a drying period of 60 hours and a fall in relative humidity of from 100% to 40%, based upon commercial practice, is described.

An automatic recording balance has been employed to study the rate of moisture loss during the drying operation and also to secure data upon the equilibrium moisture values at different relative humidity levels.

A tentative experimental technique for processing macaroni from semolina is described. Employing the selected milling and processing procedures, 19 replicate tests were made from a uniform sample of No. 1 C.W. Amber Durum wheat, and the variability of milling yield and carotene content of semolina, and of brightness, purity, and dominant wavelength for the macaroni determined as a basis for establishing criteria of the differences required for distinguishing between different samples.

Acknowledgments

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SOME PHYSICO-CHEMICAL PROPERTIES OF WHEAT FLOUR PROTEINS [1]

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Since it was discovered that the peculiar properties of the protein material in the endosperm of wheat flour were the factors enabling yeast-leavened bread to be made from wheat flour, many investigators have endeavored to discover how the proteins differ from flour to flour so that some flours have different bread-baking abilities than others.

By degrees it was discovered that although the protein substance of any material was not wholly soluble in water or in any other known solvent, that it could be fractionated by submitting the material to various solvents, and as long as the procedures were rigidly carried out, these fractions were reasonably identical in different samples. A system of classification was finally worked out which was based chiefly on differences in "solubility" and "precipitation" of the proteins in different "solvents."

Carrying this scheme of classification into the study of wheat flour proteins, it was found that flour contained small amounts of protein "soluble" in water and dilute salt solutions, respectively; that about 40% was "soluble" in alcohol, and that the remainder was "insoluble" in all of the common solvents except dilute acids and alkalis. It was believed that the properties of these last two fractions were responsible for the peculiar properties of wheat flours.

Examination of the literature shows that the protein fractions isolated by any given "solvent" may be duplicated with fair accuracy providing that the procedure is strictly adhered to in every respect, and that the flour was made from sound, undamaged wheat, and that the flours themselves were not damaged.

Nearly everyone who has investigated proteins has found that this system of classification is not specific. This is due to several reasons. First, on account of "over-lapping" solubilities, each of the fractions is partially "soluble" in the "solvents" for the other fractions.

The amount of any fraction extracted depends to some extent on the preliminary treatment of the sample and on the amount recovered by the preliminary extractions of the other fractions.

[1] Since completing this paper the author's attention has been drawn to the note by Cook and Rose on the solubility of gluten in sodium salicylate in Nature, **134**: 380–381 (1934).

The amount extracted, its physical properties, and the chemical constitution of any fraction, depends on the technique or method of extraction, particularly if the sample is not normal.

Blish (1926) came to the conclusion, from his study of the factors determining the amount and chemical composition of a protein fraction extracted by any technique, that it must be considered extremely fortunate if the prescribed technique happens to be the correct one.

Even if what are considered to be the primary fractions have been isolated they can be still further fractionated into innumerable subfractions. The glutenin and gliadin fractions themselves have been re-fractionated by various investigators using different techniques until apparently there seems to be no limit as to the number of subfractions possible.

Gortner and his associates (1927) have shown that the amount of protein extracted by salt solutions depends on the nature and the amount of salt used and not on the presence of a definite simple protein in the flour.

The work done to date indicates that the present system of classification by fractionating by means of "solvents" is decidedly an arbitrary one. Osborne (1907) emphasized the fact that we are not justified in concluding that the fractions isolated on the basis of "solubility" are definite protein individuals. From the work that has been reported by Gortner and his associates it must be concluded that protein "solubility" is not solubility in the strict sense of the term, but is peptization; and that the so-called simple proteins are not homogeneous and distinct individuals, but in reality are either heterogeneous mixtures or more probably merely fragments of a larger and more complex protein micelle, the size and properties of which depend on the nature and amount of dispersing agent present in the dispersing solution.

The fact that the present hypothesis of the protein material of any substance being composed of a mixture of several "simple" proteins is ambiguous if not meaningless, is not confined to the protein of wheat flour is shown by the work of Lustig (1930) who, by a modified technique, obtained five globulins and three albumins from blood serum; and by Sinclair and Gortner (1930) who studied the salt "soluble" proteins of seeds other than the cereals.

The most fruitful work on the nature of the proteins has been the study of their physical properties. It is well known that wheat flour protein imbibes water and behaves as a typical hydrophylic colloidal substance. Sharp and Gortner (1923) found that maximum imbibition occurred at a pH of 3 in acid suspensions. They showed that the protein fraction which could not be extracted with water was re-

sponsible for the increase in hydration capacity in the presence of acid solutions. They extracted as much as 60% of the total protein by successive extractions with distilled water without appreciably lowering the viscosity values. Johnson (1931) found that even if this amount of protein was extracted by dilute salt solutions and the salt washed from the undispersed portion, that its hydration capacity was not appreciably lowered. Apparently it is that fraction of the total protein (about 40%) which is not dispersed into small enough particles by either water or dilute KI solutions which has the greatest hydration capacity. This fraction is obviously identical with that fraction hitherto designated as "glutenin."

Experimental

In a previous paper Rich (1933) found that the amount of protein "soluble" in a normal solution of $MgSO_4$ was positively and very highly correlated with the grade of the flour as measured by the ash content of the flour.

This study was continued in order to determine what was the relation between the other fractions isolated from wheat flour and its grade. The flours were all taken from the same mill at the same time and were made from Western Canadian hard red spring wheat.

The method of obtaining protein fractions, which is similar to that described in the previous paper, was as follows:

> Six grams of flour were weighed into a flask and 150 cc. of "solvent" added. The suspension so formed was shaken at 5-minute intervals for one hour. The flask was then set aside for several hours to settle. The clear supernatent liquid was decanted and the protein determined in the regular way.

The data showing the amount of protein extracted by pure distilled water, N/2 $MgSO_4$, 10% NaCl, and N/4 NaCl solutions are given in Table I, and also as Figure 1.

The amount extracted by salt solutions varies directly with the ash content of the flour as was shown by Rich (1933). The relation between flour grade (as measured by ash content) and the fraction extracted by distilled water was not as simple. The curve is apparently rectangularly parabolic, symmetrical about 0.65% ash content. The tendency for a parabolic curve, when water-soluble protein is plotted against ash content, is also shown in the data of Jacobs (1915) and Harris (1930). Rigid interpretation of the albumins and globulins of wheat flour leads to very peculiar conclusions. If a 10% NaCl solution extracts albumin plus globulin, and water alone only albumin, then in all but the lower grades of flour there is a minus quantity of globulin present.

According to Osborne's classification, the fraction extracted by pure water contains albumin plus proteose or non-protein nitrogen. The water soluble fraction not precipitated by $Cu(OH)_2$ was also determined for some of the flours. This is supposed to be a measure of the non-protein nitrogen, although the amount obtained depends on the heavy metal used as a precipitant for the protein nitrogen. Nitrogen $\times$ 5.7 not precipitated by $Cu(OH)_2$ is apparently highly correlated with the ash content of the flour.

TABLE I

THE AMOUNT OF PROTEIN EXTRACTED FROM WHEAT FLOUR BY DIFFERENT SOLUTIONS

Laboratory number	Ash content	Total protein	Protein extracted by				Water "soluble" non-protein N × 5.7
			Water	N/2 $MgSO_4$	10% NaCl	N/4 NaCl	
	%	%	%	%	%	%	%
1	0.33	13.10	3.10	1.80	1.70	2.70	
2	.34	12.50	3.00	1.80	1.70	2.70	
3	.34	13.20	3.30	1.90	1.80	2.70	1.00
4	.36	12.70	2.90	1.90	1.60	2.60	
5	.38	12.70	2.80	2.10	1.70	2.70	
6	.39	12.90	2.70	2.00	1.80	2.90	
7	.39	13.80	2.75	2.00	1.80	2.80	
8	.41	12.60	2.65	2.00	1.80	3.00	
9	.41	13.80	2.70	2.10	1.90	2.90	
10	.42	16.80	2.60	2.10	1.90	3.00	
11	.43	12.50	2.60	2.10	2.10	3.00	
12	.43	14.00	2.70	2.10	2.00	3.00	1.00
13	.47	14.50	2.50	2.25	2.20	3.20	1.20
14	.48	17.10	2.55	2.40	2.10	3.10	
15	.50	19.80	2.80	2.30	2.10	3.20	
16	.54	12.90	2.30	2.40	2.45	3.30	
17	.54	15.80	2.50	2.70	2.30	3.40	1.30
18	.54	19.80	2.70	2.75	2.30	3.20	
19	.61	16.70	2.50	2.60	2.50	3.40	
20	.62	15.30	2.40	2.60	2.40	3.60	
21	.68	15.20	2.45	3.00	2.80	3.60	
22	.72	17.50	2.40	3.20	2.90	3.90	
23	.87	19.50	2.85	3.75	3.20	4.10	1.80
24	.90	19.20	2.90	3.30	3.20	4.40	
25	.95	20.00	2.85	3.50	3.50	4.60	1.60

The Effect of Salts on the "Solubility" of Protein

Osborne (1924) noted that it was difficult to know whether vegetable proteins were soluble in water alone or whether their solubility was due to the presence *in the seed* of minute quantities of salts, acids, or bases.

Gortner, Hoffman, and Sinclair (1927, 1929) showed that the amount of protein extracted by the alkaline halides becomes less as

the salt concentration of the dispersing agent increased. The data in Figure 1 and in Table I suggest that the decreasing "solubility" of protein in distilled water as the ash content increases might be due, as Osborne suggests, to the mineral salts in the flours.

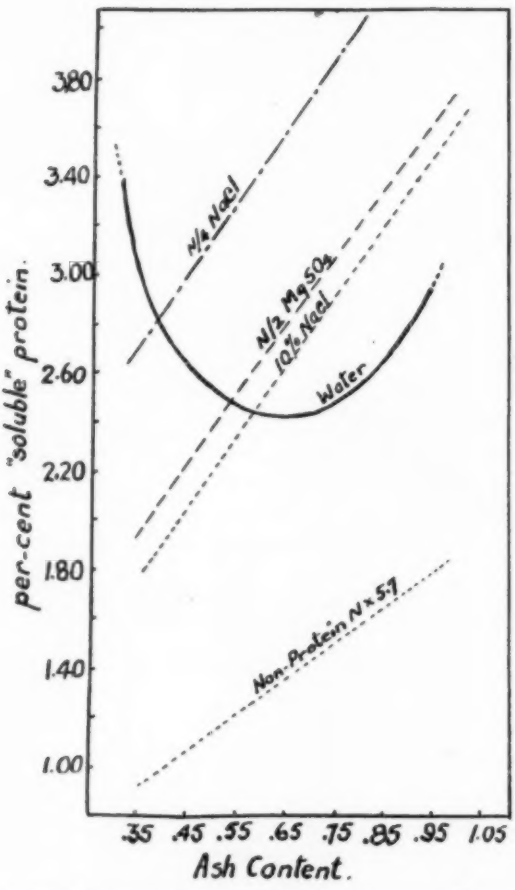

Fig. 1. The amount of protein extracted from 25 flours from the same mill but of varying ash contents, by different dispersing agents.

Accordingly, a series of flours was extracted with the commonly used salts of various concentrations. The data are given in Table II and shown graphically in Figure 2. The amount of protein extracted from flour No. 3 (ash content, 0.34%) decreased from 25% of the total protein when distilled water was used as the solvent, to much lower amounts as the concentration of the salt was increased. The curves are apparently of the exponential form.

TABLE II

THE EFFECT OF SALTS ON THE PROPORTION OF THE TOTAL PROTEIN EXTRACTED FROM WHEAT FLOURS BY SOLUTIONS OF DIFFERENT SALTS IN WATER

Laboratory number	Ash content	Normality of salt solution										
		0	N/4	N/2	N/¾	1 N	1½ N	2 N	3 N	4 N	5 N	6 N
		Percentage of the total protein extracted by NaCl solutions										
3	0.34	25.0	20.5	18.0	17.4	16.8		12.6	10.5	9.0	7.6	7.5
12	.43	16.4	21.2	20.0	19.0	17.8		14.5	13.2	11.5	10.0	9.4
17	.54	13.8	21.5	20.0	20.0	19.2		16.5	13.4	12.0	10.7	10.5
22	.72	13.8	22.2	22.2	20.8	20.0		15.5	14.7	13.3	12.2	11.5
25	.95	14.3	23.0		20.0	18.0		14.9	14.0	12.5	12.0	
		Percentage of the total protein extracted by $MgSO_4$ solutions										
3	0.34	25.0	17.3	14.4	13.5	12.0	11.0	10.1				
12	.43	16.4	19.8	18.0	16.5	14.8		13.2				
17	.54	13.8	19.0	16.9	16.0	14.8		13.2				
22	.72	13.8	21.0	18.2	17.4	16.2		13.5				
25	.95	14.3	20.2	17.5		16.0		14.0				
		Percentage of the total protein extracted by Na_2SO_4 solutions										
3	0.34	25.0	16.0	14.4	11.2	11.0	9.4	6.4				
12	.43	16.4	18.5	17.6	16.0	12.8	9.8	7.4				
17	.54	13.8	16.5	16.0	14.4	13.5	10.7	8.0				
22	.72	13.8	18.0	16.5	14.6	13.7	10.1	8.8				
25	.95	14.3	18.8	17.4	14.5	11.5	9.0	6.3				

The amount of protein extracted by distilled water decreased with the ash content of the flours in the same manner as recorded for the flours listed in Table I. However (with the exception of flour No. 3), when small concentrations of salts were used the amount extracted was greater than that extracted by water alone, the amount reaching a maximum with N/4 solutions. The amounts extracted by a single extraction, using N/4 NaCl solutions, are shown in Figure 1. After the maximum is reached the curves are similar to those for flour No. 3, the amount extracted decreasing with increasing concentrations of salt.

The similarity in the form of the curves in Figures 1 and 2 suggest that the magnitude of the amount of protein extracted by distilled water is not dependent on the amount of a definite protein individual in the flour, but on the amount of mineral salts in the flour. Interpolating the curves for water "soluble" protein in Figure 1 to an ash-free basis indicates that nearly all of the protein would be extracted by a single extraction with water alone. Apparently water extraction is a case of dispersion of part of the protein, the amount peptized depending on the mineral salt content of the flour.

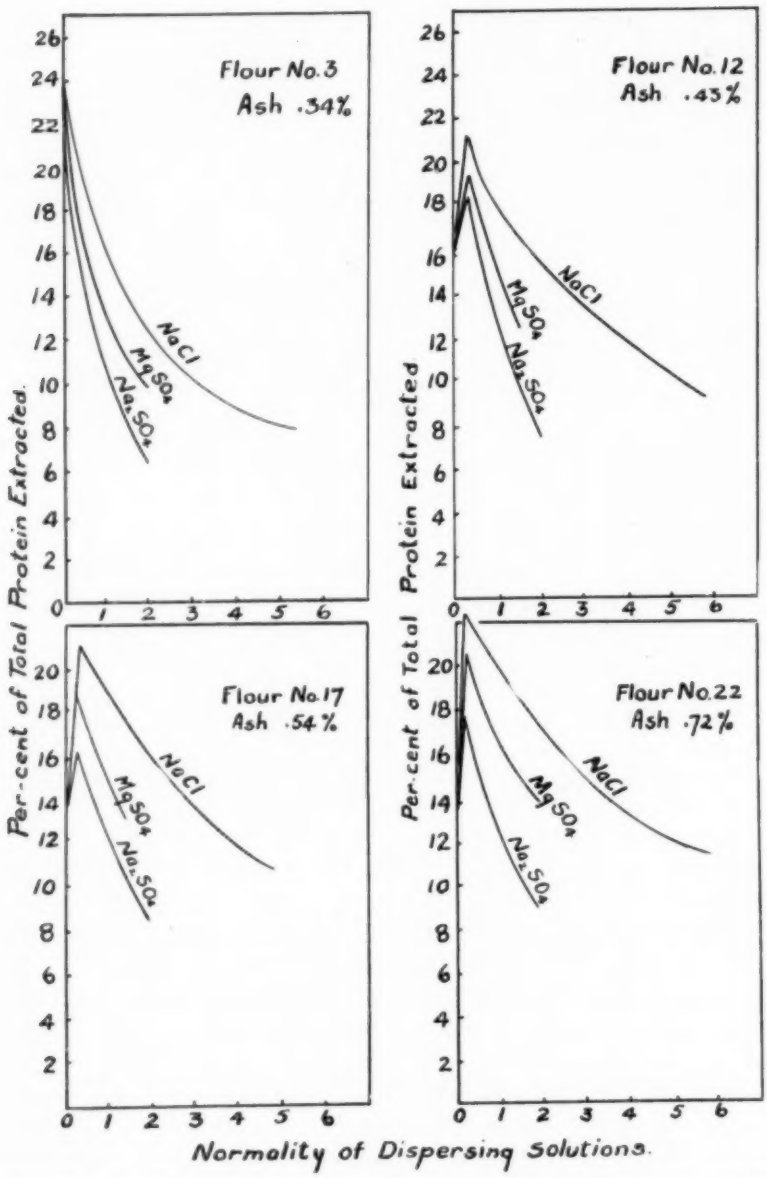

Fig. 2. The percentage of the total protein extracted from wheat flours of different grades by NaCl, $MgSO_4$, and Na_2SO_4 solutions of varying strengths.

Osborne's classification and the one adopted as tentative by the American Association of Cereal Chemists (1928) for the salt soluble fraction, is that portion extracted by a 10% NaCl solution. Some investigators have used a 1% solution, which is the concentration used

by the tentative methods of the Association of Official Agricultural Chemists (1930). The data given in Table II show that the maximum amount of protein is extracted in N/4 or about 1.5% NaCl solution. It would seem more reasonable to use this concentration than a 10% solution. The amount of "salt soluble" protein in a flour would then be approximately 22% instead of about 16%.

Bailey and Blish (1915) found that the amide-N in the fraction extracted by 1% NaCl was 16.7%, while the 10% extract had an amide-N content of about 10%. According to Osborne the amide-N content of globulin and albumin is approximately 8%. The amide-N content of fractions represented by points on the curve in Figure 2 should all be different, being highest in those fractions represented by the highest points on the curves.

From these curves it is evident that neither of the terms "albumin" or "globulin" have any definite meaning when applied to the protein of wheat flour. It is evident from these data, as well as that of Gortner *et al.*, that we are dealing with peptization of the protein micelle. The amount of protein peptized by distilled water is dependent on the small amounts of salts present in the flour itself. On increasing the concentration of the salt in the suspension throughout the range from zero to about N/4 the surface forces are altered in such a manner as to favor dispersion, following which a further increase in salt concentration causes dehydration and salting out of the protein micelle.

Osborne (1924) states that while animal "globulins" can be precipitated by saturating their solutions with $MgSO_4$, many of the vegetable proteins cannot be. This is explained by Figure 2. Extension of the $MgSO_4$ curves indicates that complete salting out would only occur with $MgSO_4$ solutions approximating $7 \times N$. This is far beyond the saturation point of $MgSO_4$. He also states that the vegetable proteins can be salted out with solutions saturated with Na_2SO_4 at 33° C. Examination of the Na_2SO_4 curves indicates that it would require about a $5 \times N$ solution to cause complete precipitation. This is beyond the saturation point at room temperature but solubility data show that this concentration can be reached at 32.4° C., which is the temperature of maximum solubility of Na_2SO_4. By actual experiment no protein could be extracted from any of the samples by solutions saturated with Na_2SO_4 at 33° C. The solubility of NaCl and $MgSO_4$ is not increased appreciably with temperature changes and thus it is impossible to salt out wheat flour proteins with these salts. The temperature of the peptizing solution appears to have no effect on the amount of protein dispersed. This is shown in Table III, which records the amount of protein extracted by various solutions at various temperatures.

TABLE III

THE EFFECT OF TEMPERATURE ON THE AMOUNT OF PROTEIN EXTRACTED BY WATER AND BY SALT SOLUTIONS

Dispersing agent	Amount of protein extracted as per cent of flour				
	Temperature				
	5° C.	10° C.	20° C.	40° C.	60° C.
	%	%	%	%	%
Water	3.2	3.2	3.2	3.1	3.1
N/4 NaCl	2.6	2.6	2.7	2.8	2.7
N/4 $MgSO_4$	2.2	2.3	2.4	2.3	2.4
N/4 Na_2SO_4	1.8	2.0	1.8	2.2	2.0

The Effect of Hydrogen-ion Concentration on the "Solubility" of Wheat Flour Proteins

The protein of wheat flour is completely dispersed in solutions of dilute acids or bases—in fact, part of the protein has hitherto been dispersed only in such solutions. No data could be found relative to what concentration of acid or base was best suited for complete and satisfactory dispersion. The concentrations used by various investigators apparently were chosen arbitrarily.

Blish and Sandstedt (1929) point out that even with dilute acid solutions part of the protein is dispersed into such large particles that they cannot be decantated or centrifuged free from the starch residue of the flour, so that the gluten must first be washed free from starch before dispersing it.

In an effort to discover what the "solubility" of wheat flour protein is for different concentrations of acids and bases, flours of widely differing grades were extracted with solutions containing various concentrations of different acids and of KOH. The method of extraction was similar to that described at the beginning of this paper. Various amounts of normal solutions of these substances were added to the 150 cc. of water before adding the flour. The results obtained are recorded in Table IV and shown graphically as Figure 3. It will be seen that a very small addition of either acid or alkali tremendously increases the amount of protein that can be extracted by decantation from the suspension.

The amount extracted reached a *minimum* value when the suspension was on the alkaline side (shown in Figure 3 as the minimum points of the curves). When the alkali concentration was increased more protein became "soluble" until at a concentration of approximately N/2/100 all of the protein was completely dispersed and could be decanted from the residue. Blish and Sandstedt (1925) used N/25/1000 NaOH solution as the minimum concentration of dispersing agent in their method for the direct determinatio n of "glutenin."

TABLE IV

The Effect of pH on the Amount of "Water-Soluble Proteins"

The Per cent of the Total Protein Extracted by Adding Varying Amounts of Normal Lactic, Acetic, and Hydrochloric Acids and Potassium Hydroxide to 150 cc. of Water Used as the Extracting Agent

Amount of acid/alkali added	Normality of the dispersing solution	Amount of protein extracted, as per cent of total protein															
		Flour No. 3 (ash content 0.34%)				Flour No. 22 (ash content 0.72%)				Flour No. 23 (ash content 0.87%)				Flour No. 25 (ash content 0.95%)			
		Lactic acid	Acetic acid	HCl	KOH	Lactic acid	Acetic acid	HCl	KOH	Lactic acid	Acetic acid	HCl	KOH	Lactic acid	Acetic acid	HCl	KOH
Cc.																	
0	0.0000	25.0	25.0	25.0	25.0	17.0	17.0	17.0	17.0	15.6	15.6	15.6	15.6	13.9	13.9	13.9	13.9
.10	0.0006	32.5	30.2	37.0	23.1	18.9	20.2	40.2	17.0	15.8	15.8	16.2	15.6	14.0	13.7	18.9	13.7
.20	0.0012	52.5	37.5	65.1	20.1	21.3	37.5	49.5	16.8	18.7	17.7	20.5	15.6	16.3	16.3	22.5	13.8
.50	0.0033	70.5	53.6	65.1	22.8	44.6	48.0	66.0	22.8	50.0	39.7	68.2	18.2	35.3	34.5	54.4	17.9
1.00	0.0067	71.9	62.0	73.9	47.0	70.3	58.3	71.4	39.0	69.8	53.5	71.9	34.0	60.7	51.2	71.5	42.5
2.00	0.0134	71.9	65.0	72.3	90.0	70.9	52.1	70.0	85.2	72.0	61.1	73.5	98.5	72.6	57.0	72.3	72.3
3.00	0.0198	71.5	64.6	71.5	99.5	71.5	64.8	71.3	99.5	73.2	63.5	76.0	100.0	71.5	59.7	71.5	100.0
4.00	0.0264	71.5	65.5	71.2	99.5	71.5	64.8	71.3	99.5	71.5	63.5	71.9	100.0	72.3	60.5	72.3	99.5
5.00	0.0330	72.0	65.5	72.0	99.5	72.0	64.4	72.0	100.0	71.5	65.0	72.5	99.3	71.8	63.6	72.6	100.0

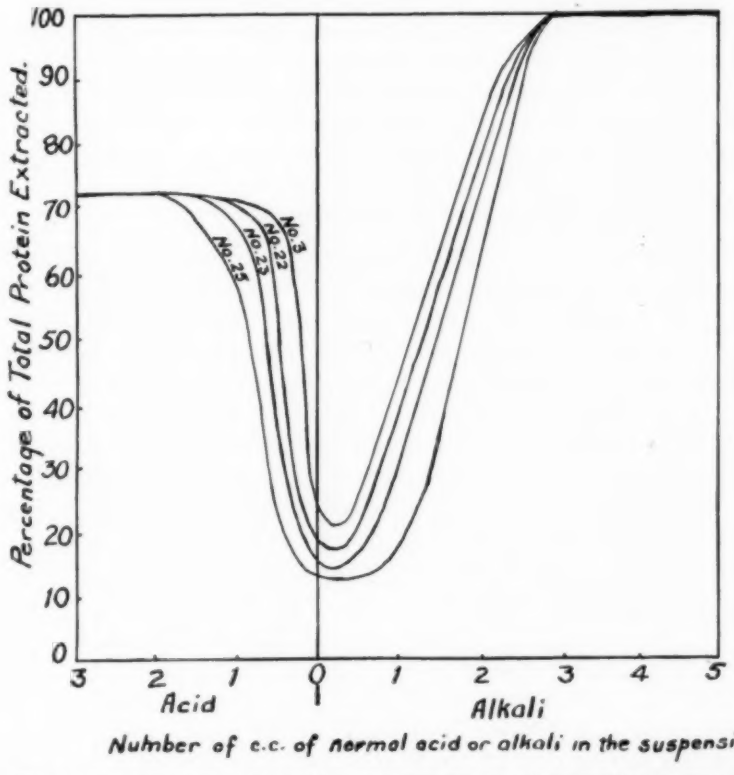

Fig. 3. The percentage of the total protein extracted by adding varying amounts of lactic, and hydrochloric acids and potassium hydroxide to 150 cc. of water used as the extracting agent.

The amount extracted from the acidic suspensions reached a maximum of approximately N/5/1000. The curves shifted slightly towards the left as the ash content of the flours increased, due to their greater buffer action. This concentration is in the region of pH 3. As no apparatus was available actual pH values could not be determined. Blish and Sandstedt (1929) used very dilute solutions of acetic acid but did not state the actual concentration they used as a dispersing agent for gluten. Larmour and Sallans (1932) found that N/7/1000 acetic acid was more satisfactory than the stronger N/7/100 solution. Hydrochloric acid was chosen because it is possible to bring the suspensions to low pH values. Lactic acid is used almost exclusively in viscosity work and acetic is the acid generally chosen in the determination of "glutenin." By single extraction the maximum amount of the total protein extracted by either lactic or hydrochloric acids was about 72%. Acetic acid solutions dispersed about 65% of the total protein leaving about 35% of protein in the residue. Grewe and Bailey (1927) found that the "glutenin" content of a series of flours by the Sharp and Gortner indirect method was about 37% of the total protein content. The fact that hydrochloric and lactic acids extract more than acetic acid may be explained by their greater imbibitional powers as was shown by Sharp and Gortner who demonstrated that the viscosity values obtained by acidifying with acetic acid were smaller than when either lactic or hydrochloric acids were used.

Appearance of the Suspensions

The appearance of the suspensions was interesting. With lactic and acetic acids the samples swelled and occupied more space than the pure water suspensions after the sample was allowed to settle for a few moments. After about N/5/1000 was reached it was difficult to decant 100 cc. of clear "solution" due to the fact that a large amount of the water was held by the swollen residue in the bottom of the flask. This residue had the appearance of a gelatinous precipitate and remained the same regardless of any further addition of acid, due to the fact, as was shown by Sharp and Gortner (1923), that the pH cannot be decreased much beyond 3 with lactic or acetic acids.

With HCl the samples continued swelling with additions of acid up to about N/5/1000. Decantation was difficult at this point. When the acidity was increased the samples no longer swelled but formed a compact, level mass at the bottom of the flask, and 125 cc. of the original 150 cc. could readily be decantated. In spite of this, no more protein was dispersed, even with an N/8/100 solution. Upson and Calvin (1916) noted that gluten loses weight and shrinks in the higher concentrations of HCl because of loss of water and not because of

"solution" of the gluten. Sharp and Gortner (1923) found that maximum viscosity of flour-in-water suspensions occurred at a pH of 3 (where "solubility" reaches a minimum), indicating that the proteins reach their maximum imbibition at that pH, after which imbibition is lowered and viscosity values drop correspondingly.

The appearance of the alkaline suspensions was decidedly different. There may have been a slight swelling at the alkali concentration corresponding to the minimum points of the curves but this was not noticeable. The residues all took the form of a flocculent precipitate and at the higher concentrations the suspensions were milky and became brownish colored. All observers state that in alkaline suspensions the proteins become disintegrated.

In other experiments it was found that on neutralizing suspensions in N/5/1000 acid, the re-precipitated gluten resembled the original gluten in appearance but that it had lost its important characteristics of elasticity, extensibility, and strength. On neutralizing weakly alkaline dispersions of gluten, the re-precipitated gluten had none of its original appearance nor its physical properties.

The Influence of pH on the Amount of Protein Extracted by Salt Solutions

Gortner, Hoffman, and Sinclair (1927, 1929) found that the peptization of wheat flour proteins by salt solutions was apparently not affected by the pH of the suspension.

The amount of protein extracted by distilled water is decidedly influenced by its pH. Accordingly, several samples of flour were dispersed by $MgSO_4$ solutions containing various amounts of acid and alkali. The data given in Table V show that the amount extracted by $MgSO_4$ solution is very little influenced by the acid or alkali present unless they are present in relatively large amounts.

TABLE V

THE EFFECT OF SMALL AMOUNTS OF ACIDS OR ALKALI ON THE AMOUNT OF PROTEIN EXTRACTED FROM WHEAT FLOURS BY N/2 $MgSO_4$ SOLUTIONS

Amount of acid/alkali added	Flour No. 3	Flour No. 22	Flour No. 25
Lactic acid, cc.	%	%	%
None	1.90	3.20	3.60
0.10	2.00	3.20	3.60
0.50	2.10	3.20	3.70
1.00	2.10	3.30	3.80
KOH, cc.			
0.10	2.00	3.20	3.70
0.50	2.10	3.30	3.70
1.00	2.20	3.40	3.80

When is Extraction Complete?

In all the foregoing experiments a single extraction was made. This is the procedure given in the tentative methods of the A.O.A.C. and the A.A.C.C., and apparently that intended by the committee which adopted the definition of the proteins. Most investigators have found it necessary to make several extractions in order to insure that extraction is complete. Accordingly, a sample of flour (No. 3) was successively extracted with several dispersing agents. The data are given in Tables VI and VII, and shown graphically in Figure 4.

TABLE VI

THE AMOUNT OF TOTAL PROTEIN EXTRACTED FROM FLOUR NO. 3 BY SUCCESSIVE EXTRACTIONS WITH DISTILLED WATER

Number of extraction	6 g. flour per 150 cc. water	6 g. flour per 600 cc. water
	Amount extracted (per cent total protein)	Amount extracted (per cent total protein)
	%	%
1	24.1	24.5
2	15.9	13.6
3	9.2	9.5
4	8.0	8.8
5	3.0	2.7
6	1.2	1.0
7	0.6	0.2
Total amount extracted	62.0	60.3

TABLE VII

THE AMOUNT OF TOTAL PROTEIN EXTRACTED FROM FLOUR NO. 3 BY SUCCESSIVE EXTRACTIONS WITH DIFFERENT SOLUTIONS

Number of extraction	Dispersing agent			
	10% NaCl	Water	N/4 KI	N/4 Sodium salicylate
	%	%	%	%
1	16.9	24.1	38.5	85.0
2	9.0	15.9	17.7	14.8
3	4.6	9.2	11.5	0.0
4	3.9	8.0	5.6	
5	2.0	3.0	1.2	
6	1.1	1.2	0.6	
7	0.3	0.3	0.3	
Total amount extracted	37.8	61.7	75.4	99.8

In each case several extractions were necessary to disperse the maximum amount. It is interesting to note that approximately 60% of the total protein can be extracted by successive extractions with

water. Sharp and Gortner (1923) noted that by a single extraction with water they extracted most of the water-soluble salts from their samples, and that by successive extraction they were able to extract

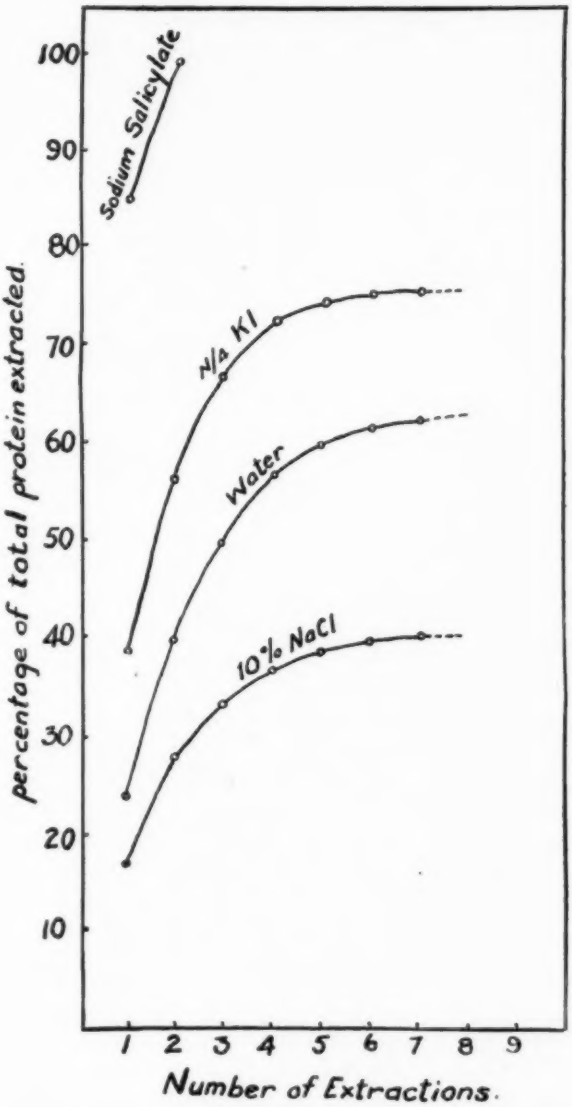

Fig. 4. The percentage of the total protein extracted from flour No. 3 by successive extractions of different solutions.

approximately 60% of the total protein without appreciably decreasing the viscosity of the remaining suspension from the value obtained after the first extraction. From this they concluded that the remaining

40% of the protein, which they concluded to be the "glutenin," was responsible for the increased imbibition of water. Johnson (1931) extracted as much as 57% of the total protein with various alkali halide solutions without decreasing very much the viscosity of the remainder.

Isolation of protein fractions by means of single extractions, using any dispersing agent, is apparently of little meaning.

It is evident from the present study and from the work of other investigators that the protein of wheat flour is *not* a mixture of individual simple proteins. The number of fractions which can be isolated are apparently limitless, depending only on the dispersing agent and the method used. Sandstedt and Blish (1933) visualized a few fractions represented graphically as *broad plateaus* at different levels connected by sloping terraces; although they state in the same paper that "there is no established limit as to the possible minor variations in the number . . . of protein fractions by this method."

The more probable idea is that of a *continuous* exponential curve which would illustrate that a fraction could be isolated representing *any point* along this continuous curve, depending on the procedure of extraction.

In search of a dispersing agent which will disperse all of the protein, dilute acids and alkalis have been most commonly used. Alkalis, even in very dilute solution, have the disadvantage that they alter the chemical structure of the protein so that when the protein is re-precipitated or re-coagulated it no longer has either the chemical nor the physical properties it originally had. Larmour and Sallans (1932) found that dilute acids have a similar effect if the concentration is too great or the time of extraction is too long, and in any case the re-precipitated protein does not resemble the original in that it loses most of its elasticity and strength. Dilute acids also have the disadvantage that the protein cannot be dispersed into particles small enough to be removed from the starch and it is necessary to start with washed gluten.

It is evident then that dispersing wheat flour protein by removing it from its iso-electric point is of little value in studying protein quality on account of possible alteration of its chemical structure and because denaturation cannot be avoided.

Cook (1931) and Cook and Alsberg (1931) used urea solution as a dispersing agent. This reagent has the disadvantage that a relatively concentrated solution must be used and that starch as well as protein is dispersed, so that the protein sol cannot be separated from the sample unless the gluten is first washed from the starch. The washing process and the action of the urea solution provides the opportunity of denaturation of the protein.

Gortner, Hoffman, and Sinclair (1927, 1929) showed that the amount of protein dispersed by salt solutions depends on the nature and the concentration of the salt used as a dispersing agent. By making three extractions with normal KI solution they were able to extract from different flours from 55.4 to 73.9% of their total protein. From Table I and Figure 1 it would be assumed that the maximum amount possible to extract would occur with a N/4 solution. In Table VII it was shown that 73.3% of the protein from flour No. 3 could be extracted by successive extraction with N/4 KI solution.

It was found in these studies that, as the amount of total protein dispersed becomes larger, it becomes more difficult to check results. Rich (1933) pointed out that this was also true of the work of Gortner *et al.* (1927, 1929), and of Harris (1930), because the correlation between ash content and the amount of protein peptized decreased as the amount of protein peptized increased. This is no doubt due to the fact that the fractions which first split off the original particle (*e.g.*, with KF) are relatively small and readily remain in suspension. The fractions splitting off become increasingly larger as more are split off. The larger the particles the more difficult it is to extract them by either decantation or centrifuging because they tend to become entangled with the starch and non-peptized fraction and so join the residue at the bottom of the flask, hence the difficulty of always extracting the same amount. This is in accordance with the view of Blish and Sandstedt (1929), and Sandstedt and Blish (1933) who believe that there is a wide variation in the size of the particles. The largest non-fiterable particles are those which have been given the name "glutenin."

Because it was found impossible to extract all of the protein from a flour sample an effort was made to disperse washed gluten in KI solutions. Unlike dilute acid or urea, it was found that part of the gluten could not be dispersed in any strength of solution of KI.

Gortner, Hoffman, and Sinclair (1927, 1929) showed that the peptizing effect of salt solutions on wheat flour proteins arranged themselves in the order of the Hofmeister's series, *i.e.*, $F < SO_4 < Cl < Br < I$. The problem seemed to be to discover what were the next ions in the series, because with one of the ions further to the right in the series it should be possible to completely disperse the protein so that the protein sol could be extracted as a whole and be studied as a whole.

Mangels and Bailey (1933, 1933a) working with starches showed that certain salts had the effect of swelling or gelatinizing starch and that the effect of various salts could be arranged in the same Hofmeister's series. They added two more ions to the series making the series now $F < SO_4 < Cl < Br < I < CNS <$ salicylate $< OH$.

For obvious reasons KCNS could not be used and KOH alters the protein molecule. Accordingly, several flours were extracted with N/4 sodium salicylate solution. It was found that about 85% of the total protein could be extracted with a single extraction and that by a second extraction all of the protein could be extracted from the sample. There was very little swelling and dispersion of the starch at this concentration. At higher concentrations the protein could not be isolated because the starch was also dispersed.

Although all of the proteins cannot be extracted from flour by dilute acid solutions it is possible to completely disperse washed gluten to an opalescent sol. In the same manner it was found that washed gluten could be completely dispersed in N/4 sodium salicylate solution.

If gluten is dispersed in dilute acid solution (to about pH 3) and brought back to the iso-electric point, it coagulates again. Although it resembles the original gluten to some extent, it has lost most of the important physical properties of the original gluten. The completely dispersed gluten in N/4 sodium salicylate solution was placed in a cellophane bag and dialyzed against tap water for several hours. Gluten was formed again which, to the sense of feel, possessed all of the properties it originally had before dispersion.

Discussion and Conclusions

The present view of the existence of five distinct protein substances in wheat flour is not justified by recent work. By arbitrarily choosing the methods of isolation, five protein fractions may be obtained. If, however, the dispersing agent or the method of isolation is changed, even slightly, there is no established limit as to the *number*, *quantities*, and *character* of the protein fractions that can be isolated. This fact is well demonstrated by examination of the literature of the research on wheat flour proteins.

The work of Gortner and his associates has shown that the term "globulin" has no meaning when applied to the cereals and to several other seeds. Doubt has also been thrown on the existence of "gliaden" as a distinct protein. Blish and his associates by their work on "glutenin" have shown that its existence as an individual chemical entity is problematical.

Apparently all the protein molecule is not dispersed to the same degree or with the same ease. The particles split off are larger as dispersion continues. The fractions dispersed by the agents of lowest dispersing power are readily separated from the starch and non-peptized protein. As greater amounts are peptized by agents of greater

peptizing power it becomes more difficult to secure close checks in the amount extracted, due most probably to the relatively large particles becoming entangled in the residue. In the case of acid dispersions, this is quite evident because the particles become swelled by increased imbibition and at the point of maximum imbibition it is impossible to seperate all of the dispersed particles from the starch by decantation. The larger particles are apparently those which have hitherto been designated the "glutenin" fraction.

Apparently there are no well-defined boundaries by which the total protein can be classified into definite fractions. Differentiation of particle size must be gradual, which would be shown graphically in the form of a continuous curve.

From these considerations it is apparent that the protein of wheat is a single individual and that knowledge of the nature of differences in protein "quality" between "strong" and "weak" flours can best be solved by studying the protein *as a whole* instead of splitting it into arbitrary fractions and studying each fraction separately.

The study of wheat flour protein has been difficult because it has been impossible to get the protein molecule as a whole into solution without causing irreversible chemical alteration or denaturation. By using a dilute solution of sodium salicylate, it is possible to disperse all of the protein of wheat flour, apparently without alterating it in any way.

Sodium salicylate has the advantage that the protein can be studied at its iso-electric point. This cannot be done when dilute acid is used as the dispersing agent. Urea has to be used at a relatively great concentration with the possibility of denaturation. It also increases imbibition. Because urea at the concentration which must be used also gelatinizes the starch granules, washed gluten must be used as a starting point. Dill and Alsberg (1924) have pointed out the difficulties of the gluten-washing process. Dilute sodium salicylate solution does not gelatinize the starch appreciably at the concentration necessary for protein dispersion so that the protein can be extracted from the flour without the danger of altering the properties of the protein or the loss of protein by washing technique.

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DETERMINATION OF MILK SOLIDS NOT FAT IN BREAD

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In recent years manufacturers have been greatly interested in producing foods of superior nutritive value. The acknowledged superior nutritive value of milk has led to its use in many products. Thus the baking industry has been using increasing quantities of milk in various forms. From the fact that milk solids have been used so extensively in bread has naturally evolved an interest in determining the quantity of milk contained in various breads. The method described in this paper is adapted to the determination of milk solids not fat, regardless of the type of milk or milk products which have been used in the bread.

Since lactose is the largest constituent of milk solids not fat and the most nearly constant in percentage, it has been taken as the index of the percentage of non-fat milk solids present. The average percentage of lactose in milk solids not fat is 50% and this has been taken as the factor for converting the % lactose to percentage of milk solids not fat.

Review of the Literature

In reviewing the literature on this subject, a number of papers were found. Work done by Coe (1928), Davis (1928), and Magraw and Sievert (1935) on the determination of milk products in feed suggested the application of their methods to milk breads. Other investigators (see Allen, 1923; Schirt and De Jong, 1925; and Snethlage, 1926) have worked directly on the bread. In most of the cases reported in the literature the estimation of the milk solids content was based on the determination of lactose. Hartmann and Hillig (1935), however, base the milk solids content on the citric acid content. Recently a paper on the estimation of milk solids in bread by the determination of butterfat was presented by Munsey before the Washington meeting of the A. O. A. C.

[1] Under direction and counsel of E. C. Thompson, Martin Kloser, and A. H. Johnson, who comprise the Standards Committee of the American Dry Milk Institute, Inc.

Difficulties Encountered

One of the main difficulties encountered in developing a method for the determination of milk solids not fat in bread was occasioned by the variation in bread formulas and the manner in which they are expressed. Percentages of ingredients in a bread formula are expressed as the ratio of that ingredient to the flour, rather than its portion of the total formula weight. Thus, a formula calling for 6% milk solids not fat really means 6 lbs. of milk solids not fat to every 100 lbs. of flour. Consequently, when the other ingredients are included, the actual percentage on the moisture-free basis will be somewhere in the neighborhood of 5.55% to 5.75%.

The variations in formula also introduce a problem in that one formula may have several more ingredients than another formula or it may have different percentages of the same ingredients. This fact may cause a discrepancy of from $\frac{1}{4}$% to $\frac{1}{2}$% in the calculated amount of milk solids not fat actually present in the bread. Consequently, due to these variations some tolerance must be allowed in estimating the percentage of milk solids not fat from the determined percentage based on the lactose content.

Another factor encountered was the loss of the lactose due to fermentation. Since the method is based on the determination of lactose, this factor cannot be disregarded. This fermentation loss will be taken up later under the topic "Experimental."

The fermentation of all soluble sugars and sugar derived from starch had to be completed before any accurate estimate of the lactose could be accomplished. The high starch content was finally removed through precipitation with alcohol and by the use of the starch-splitting enzyme diastase.

Apparatus and Reagents

Centrifuge and suitable sediment tubes
1G4 Jena fritted glass filtering crucible
Animal diastase (Armour & Co., Pharmaceutical Dept., Chicago, Ill.)
Baker's yeast
Solution of saturated neutral lead acetate
Solution of 5% mercuric chloride
Solution of 20% phosphotungstic acid
Hydrogen sulphide
95% ethyl alcohol.

Method

Cut the crust from a loaf weighing at least a pound. Slice or break up the remainder sufficiently to allow uniform drying. This is done by exposing to air until sufficiently dry to crumble. Crush finely with

a rolling pin. Determine moisture content of this air-dried sample for use in calculation. Weigh a 15 g. sample into a 300 cc. volumetric flask, add 200 cc. of distilled water and digest in a hot water bath for a period of 30 minutes, with occasional shaking. Cool the solution, fill to volume, then centrifugalize and remove insoluble material.

Place 150 cc. of this solution in a 300 cc. flask, add 150 cc. of 95% ethyl alcohol and make up to volume with distilled water. After standing for 10 or 15 minutes, remove the flocculant precipitate by centrifugalization. Boil 250 cc. of the resulting liquid over a hot plate until no alcohol odor is perceptible. Transfer the solution to a 200 cc. volumetric flask with hot distilled water keeping the total volume below 150 cc. Add 0.25 g. animal diastase and place in a constant temperature bath of 53°–55° C. for 25 to 30 minutes. Then place the flask in a boiling water bath for 15 minutes. Cool the flask and add another 0.25 g. of animal diastase and repeat the inversion and subsequent heating as before. After the flask has been cooled to room temperature add 1½ to 2 g. of baker's yeast and plug the flask with non-absorbent cotton.

After fermentation at 26½° to 29° C. (80–84° F.) for a period of 17 to 18 hours make up to volume and centrifugalize. Boil down 190 cc. of the centrifugalized liquid to 25 or 50 cc. and wash into a 100 cc. volumetric flask with the aid of hot distilled water. Add 10 cc. of saturated neutral lead acetate solution, make up to volume and remove the resulting precipitate by centrifugalization. To 75 cc. of the filtrate in a 100 cc. volumetric flask add 2½ cc. of a 5% solution of mercuric chloride and allow to stand 25 to 30 minutes with repeated shaking. Then add 10 cc. of a 20% solution of phosphotungstic acid. Make up to volume with distilled water and remove the precipitate by centrifugalizing. If the resulting solution is not clear after centrifugalization, it should be filtered through a dry filter paper before carrying further. Saturate the resulting liquid with H_2S gas and filter. Next pipette 50 cc. of clear, colorless solution into a 300 cc. Erlenmyer flask and boil until no H_2S gas is given off. Make up the volume to 50 cc. and determine lactose by the Munson & Walker method. (Consistently better results are possible with a Jena fritted glass filtering crucible 1G4 than with an asbestos mat in a Gooch crucible.) After thoroughly washing the precipitate, dissolve in 5 cc. of hot 1 : 1 nitric acid and determine the copper by the volumetric sodium thiosulphate method. The milligrams of lactose are interpolated from the Munson & Walker table (see Official and Tentative Methods of Analysis of the A. O. A. C., p. 515 (1930)).

Calculation

The grams of sample in the final aliquot are determined by the equation: (1) 15 grams $\times \frac{150}{(300-13)} \times \frac{250}{300} \times \frac{190}{200} \times \frac{75}{100} \times \frac{50}{100} = 2.332$ grams. The fractions are the dilutions in the volumes of the filtrate used in each case. The expression (300–13) is the original volume corrected for the volume of the precipitate. The percentage of lactose is calculated by means of formula No. 2: (2) $\frac{(\text{grams of lactose} - .006)}{.96 \times 2.332} \times 100 = \%$ of lactose in the bread. In this formula .006 is the correction for blank, .96 represents the lactose fermentation loss and 2.332 is the number of grams of dry substance represented in the aliquot.

The percentage of milk solids not fat may be calculated on the moisture-free basis by the following formula:

(3) $$\frac{(\text{grams of lactose} - .006)}{(.48 \times 2.332)(100 - \%\ \text{moisture})} \times 100$$

$= \%$ milk solids not fat on moisture-free basis in bread.

Experimental

Preparation of samples. The samples which were used in developing this method were those which had been picked up in the field and baked in commercial plants. Samples were also made in the laboratory. All samples were made under the supervision of the bakery service men of this Institute and followed the regular commercial formula.

Protein elimination. A number of different protein precipitants were tried, both singly and in combination, before a suitable one was finally adopted. The combination which was adopted does not entail any close manipulation of conditions such as acidity, temperature, pH, etc., and results in a crystal clear, colorless solution.

It is important to have a solution slightly acid before the protein precipitation, for it has been found that with an alkaline solution lactose may be lost in the procedure.

Fermentation. During the experimental work many different fermentation temperatures and times were tried on breads which were not previously treated for the inversion of starch. It was found that a temperature of about 26° C. for a period of 40 to 48 hours gave a complete fermentation of the starch present but also caused a high lactose loss. This lactose loss also seemed to vary quite widely in various samples of bread, possibly due to bacterial action. However, after the inversion of the starch (which has been outlined in the procedure) fermentation was accomplished at a temperature of $26\frac{1}{2}$ to 29°

C. in 17 to 18 hours, and gave a much lower lactose loss and more comparable results.

In judging the proper fermentation temperature, blank breads were used and varying amounts of milk solids not fat added. When the averages of the results obtained on the different quantities of milk solids not fat added to the blank bread were plotted against the actual milligrams of the lactose placed in the blank bread, they gave a straight line, as shown in Figure 1. The average deviation of the results of the

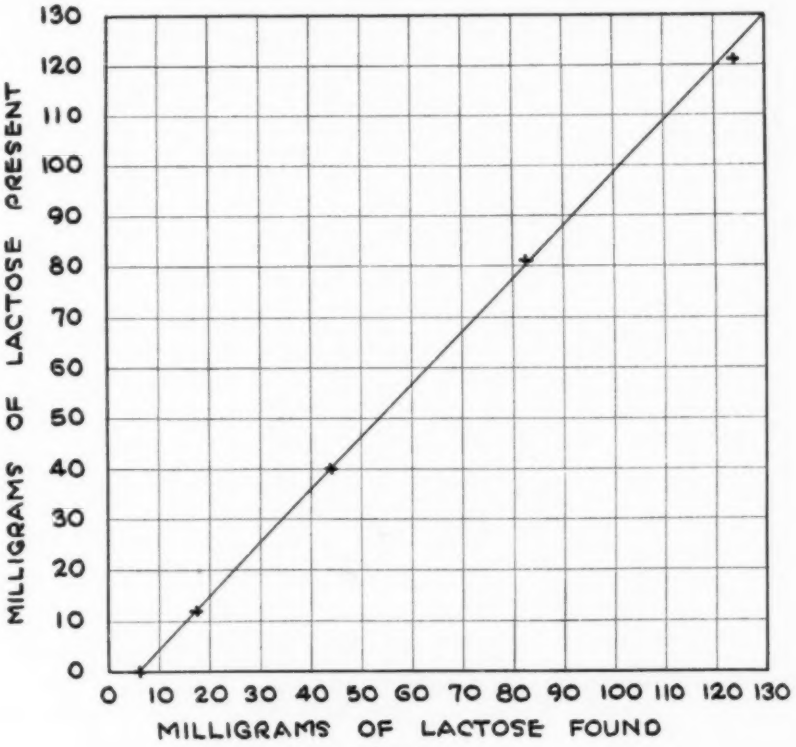

Figure 1.

different quantities of milk solids not fat added to the blank bread was considerably less than a plus or minus 2 milligrams of lactose. From this graph the conclusion can be drawn that the fermentation loss of the lactose during the procedure of fermentation is constant and gives reproducible results. It also shows that the analytical procedure is such that it allows for an accurate estimating of the lactose present, at least as accurate as one part per hundred.

After the above points were clarified, the question arose as to whether the lactose might be determined accurately if placed in the bread during the baking process. Laboratory loaves were then made

containing 0, 2, 4, 6, 8, 10, and 12% of dry milk solids, using the sponge method comparable to the commercial bakeshop formula. After these breads were carried through the above procedure and averages made on the individual results for the varying percentages of milk solids not fat, the results were plotted, using the same coordinates as shown in Figure 1, namely, milligrams of lactose determined against milligrams of lactose added to the bread. These results are shown as Figure 2.

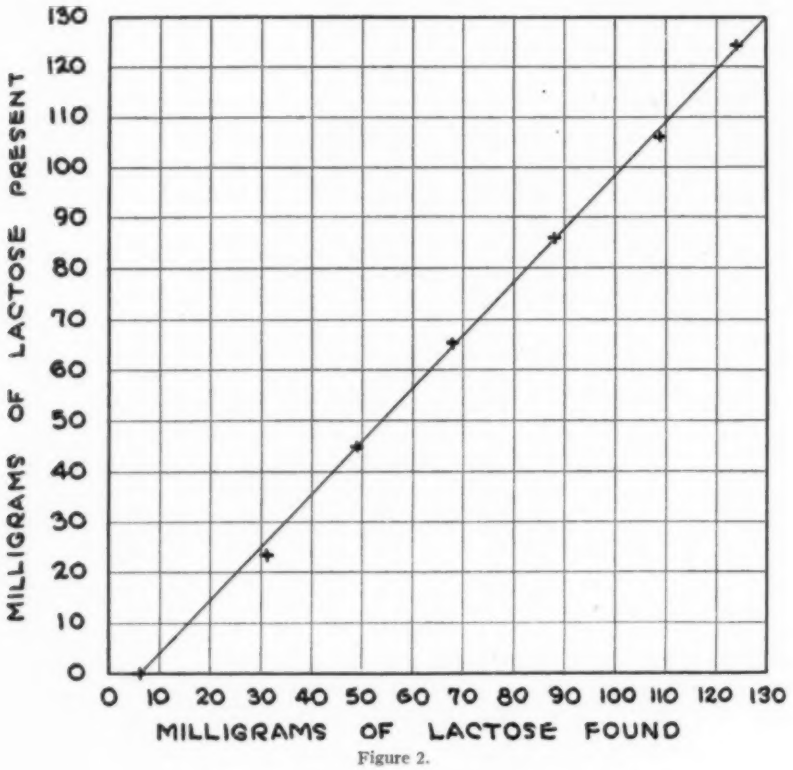

Figure 2.

When the equation for the two individual curves was caluclated, it was found that the two equations were identical. The equation representing the two curves is $\frac{x - 6}{.96} = y$. X equals the amount of lactose determined, y equals the amount of lactose present in the aliquot taken for analysis. Since these two equations are identical and the two curves might be superimposed, the conclusion may be drawn that fermentation in bread has no effect on the lactose and that lactose loss is encountered only during the analytical procedure. These results also make it justifiable to say that the fermentation in the method has a constant and reproducible effect.

Lactose Determination

It was necessary to try several different methods for the determination of lactose. From this experimental work, it was found that the volumetric thiosulphate method gave more accurate results than direct weighing of cuprous oxide. More satisfactory checks were also obtained by using a Jena fritted glass crucible, rather than a Gooch crucible with an asbestos mat, apparently due to better retention of the copper oxide. The findings on this were substantiated by several workers in other laboratories. Colorimetric methods were not studied in this work.

Corrections

In several steps of this procedure, precipitates occur which appear quite voluminous. It was found on measuring these precipitates that the residue from the first extraction of the material was 12 to 13 cc. In all of the following precipitates the insoluble material was so small that it was not taken into consideration in the correction. This correction has been made in formula No. 1 under the first dilution factor. In calculations made from Figures 1 and 2, it was found that the lactose fermentation loss was 4% or, in other words, 96% of the lactose added was recovered. This lactose loss has been included in formula No. 2 under the factor .96. It was also found that milk-free bread gave an average of 6 mg. of lactose as a blank. This blank has also been included in formula No. 2 under the factor of .006. If it is desired to have the results expressed in percentages of milk solids not fat, the assumption must be drawn that milk solids not fat contain 50% of lactose on an average, and since we have a 4% fermentation loss only 96% of this lactose would be determined. From this, equation No. 3 has been derived which includes calculations for the results to be expressed on the moisture-free basis.

Application of Results

Due to the great difference in bread formulas and also because formulas are based on weight of the flour, a marked variation may be encountered in results from the same percentage of milk solids not fat. Therefore, it is necessary to make minimum and maximum limits for the various percentages of milk solids not fat.

These variations were deduced from the determined percentages on known samples of bread, and also on the calculations from the known formulas, allowing for 13% baking losses.

Percentage given (flour basis)	Minimum percent milk solids not fat in crumb	Maximum percent milk solids not fat in crumb
1%	.8	1.2
2%	1.6	2.1
3%	2.5	3.0
4%	3.4	3.9
5%	4.2	4.6
6%	5.1	5.6
8%	6.8	7.3
10%	8.5	9.1
12%	10.0	10.8

Results

The table below shows a few of the results which were obtained on bread samples made especially for this work, and also those which were obtained from commercial runs.

The determined percentages show that calculated variations which were listed above cover the variations on the same percentage of milk solids not fat on the flour basis.

	Percent milk solids not fat used (flour basis)	Percent milk solids not fat (moisture-free basis)	Type of milk used
1	none	none	
2	none	none	
3	2%	1.76	Dry milk solids not over 1½% fat
4	2%	1.45	" " " " " " "
5	3%	3.13	" " " " " " "
6	4%	3.59	" " " " " " "
7	4%	3.79	" " " " " " "
8	6%	5.27	Combination of condensed milk and dry milk solids not over 1½% fat
9	6%	5.57	Combination of condensed milk and dry milk solids not over ½% fat
10	6%	5.31	Dry milk solids not over 1½% fat
11	6%	5.13	" " " " " " "
12	6%	5.59	" " " " " " "
13	6%	5.53	" " " " " " "
14	6%	5.79	" " " " " " "
15	6%	5.40	" " " " " " "
16	2.65%	2.44	Sweetened condensed milk. Combination of condensed skim and dry milk solids (not over 1½% fat)
18	12%	9.88	
19	12%	9.60	
20	12%	9.55	
21	12%	10.10	
22	12%	10.37	

It is realized that this method is fairly long, and entails a considerable amount of work and time. We believe, however, that it has been simplified as much as possible.

Summary

A method has been developed for the determination of milk solids not fat in bread, using lactose as the determining factor.

This method has been shown to be accurate to a degree of ± .50% of milk solids not fat.

Corrections for blanks and losses have been worked out and incorporated in the method.

The method entails no exceptionally specialized laboratory technique.

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THE RELATION BETWEEN THE VISCOSITY OF NON-SWEETENED CONDENSED SKIMMILK AND BAKING QUALITY

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Introduction

The baking industry uses considerable quantities of non-sweetened condensed skimmilk in breadmaking. The bakers who are familiar with the so-called superheated condensed skimmilk usually prefer this product to the so-called plain condensed. Both of these milks contain 30% of skimmilk solids. The superheated condensed skimmilk differs from the plain condensed product in that it has been subjected to a heat treatment after condensing. This heat treatment usually consists in blowing live steam into the milk at the end of the condensing operation, the application of steam being continued until the viscosity of the condensed milk increases very markedly or until a certain temperature is reached at which the milk is held until the increase in viscosity occurs. The temperature to which condensed skimmilk must be raised or the time that it must be held at the certain temperature differs considerably for milks in various parts of the country and for milks produced in various seasons of the year, hence, it is not possible to set down definite directions for the superheating procedure. The superheating of milk requires experience and judgment on the part of the operator. If the condensed milk is heated to too high a temperature or if it is held too long at the high temperature, the milk "breaks" and becomes filled with small curds or in the extreme case the casein may separate out entirely to give a curd and whey.

The danger of carrying the superheating procedure too far obviously makes plant operators very careful and sometimes the heating is stopped before the viscosity has been increased to the desired point. The absolute maximum in viscosity is reached just before the milk has attained its "breaking point" hence the unwillingness to approach too closely to this point. Because of these conditions the viscosity of superheated condensed skimmilk shows some variation. Now the baker has been accustomed to judge the quality of superheated condensed skimmilk by its viscosity. If the viscosity of the milk is high, it has been his experience that its baking quality would be satisfactory.

This has also been our experience when laboratory baking tests have been conducted with such milks of high viscosity. The baker has also been prone to condemn superheated milks which are not of high viscosity. While laboratory experience with some milks of low viscosity has indicated that they are of unsatisfactory baking quality, many of them have also been found to be of satisfactory baking quality. Thus the viscosity of a superheated condensed milk does not necessarily indicate its baking quality. This agrees with the observation of Skovholt and Bailey (1931) who found that the viscosity of a skimmilk powder suspension was not a reliable index of the baking quality of the powder. Thus milk which had been held at 77° C. for 30 minutes prior to the powdering operation yielded bread of just as high score as did powder produced from milk held at higher temperatures although the viscosities of the milks held at the higher temperatures were markedly above those milks held at 77° C.

Because of the confusion in the relationship which appeared to exist between the viscosity of superheated condensed milk and baking quality, the work to be reported in this paper was done. It is believed that bakers do not necessarily want a superheated condensed milk of exceedingly high viscosity as milk of high viscosity is difficult to remove from the milk cans and permits too great losses in milk solids unless washing of the cans is resorted to.

Experimental

The Effect of Heat Treatment on Viscosity and on Baking Quality

Work which has been done on the use of milk in breadmaking has indicated that a heat treatment of the milk is necessary in order that satisfactory baking results be obtained. Thus Greenbank, Steinbarger, Deysher, and Holm (1927) reported that milk that had been heated for a sufficient time at temperatures above 75° prior to condensing showed distinct improvement in baking properties as compared with non-heated milk or milk heated at low temperatures. These investigators note a binding action on the dough when milks which had not been subjected to sufficient heat treatment were used. As heating of the milk probably effected a coagulation of the albumin, they suggested that albumin had an inhibitory effect on baking quality and its coagulation by heat removed this inhibitory effect. Evidence that coagulation of albumin by heat operated to improve the baking quality of milk was also presented by Allen and Bell (1931).

As has already been stated, different lots of superheated condensed skimmilk differ in viscosity from time to time due in part to the variability with which milks react to equivalent heat treatment and in

part to difficulties of the plant operator to determine exactly when to terminate the superheating treatment. Table I records the variability in viscosity and in other properties of a series of superheated milks collected from various sections of the country for this study.

The viscosities were determined with the MacMichael viscosimeter operating at 40 revolutions per minute. A No. 30 wire and the cylindrical 1 cm. bob were used. The temperature of the superheated skimmilk was 27° C. when the determinations were made and the quantity of milk in the cup was 160 grams.

A study of the data in Table I shows that there were very consider-

TABLE I

THE VISCOSITY AND BAKING PROPERTIES OF VARIOUS SUPERHEATED CONDENSED SKIMMILKS

Sample No.	Viscosity at 27° C.	Loaf volume of bread baked with the milk	General characteristics of the bread
	° *MacM.*	*Cc.*	
5699	36	1830	unsatisfactory
5705	117	1870	satisfactory
5706	79	1955	satisfactory
5707	135	1885	satisfactory
5709	58	1865	satisfactory
5719	39	2015	satisfactory
5735	50	2090	satisfactory
5736	98	2050	satisfactory
5814	128	1865	satisfactory
5816	89	1875	satisfactory
5818	168	1880	satisfactory
5819	53	1875	satisfactory
5823	90	1960	satisfactory
5824	60	2010	satisfactory
5827	107	1995	satisfactory
5837	64	1965	unsatisfactory
6185	69	2140	satisfactory
6250	64	2175	satisfactory
6272	87	2005	satisfactory

able differences in viscosity. The baking results with these milks were satisfactory except in two instances. These instances are among the low group in viscosity. However, low viscosity does not necessarily indicate inferior baking quality. It has been found that it is adequate heat treatment that determines baking quality and that high viscosity is more or less incidental. Thus, it is quite possible to give adequate heat treatment to a superheated condensed product without imparting a marked increase in viscosity. Several experiments were conducted to clarify this relationship. Prior to condensing, a batch of skimmilk was held at 85° C. for 30 minutes and then condensed and part of the batch accorded the usual superheating treatment.

Such milk did not attain the high viscosity characteristic of the usual superheated product, yet it had very satisfactory baking properties. In Table II are given data on a series of condensed skimmilks which were prepared in such a way as to vary in viscosity and yet give satisfactory baking results. These results are also shown in the series of photographs in Figure 1. Bread No. 13 prepared with superheated milk was very satisfactory as was also the bread prepared from the milks variously heat-treated. Thus milk No. 15 was accorded no superheating treatment as such but was held at 85° C. for 30 minutes. The superheating of this preheated milk in exactly the same manner as milk No. 13 was superheated, increased the viscosity only slightly

TABLE II

THE VISCOSITIES AND BAKING RESULTS OF PLAIN, PREHEATED, SUPERHEATED, AND HOMOGENIZED CONDENSED SKIMMILK

Sample No.	Description	Viscosity at 27° C.	Loaf volume of bread baked with the milk	General character of the bread
		° *MacM.*	*Cc.*	
13	Regular superheated condensed skimmilk	65	2035	satisfactory
14	Superheated condensed skimmilk (prepared from skimmilk) which had been held at 85° C. for 30 minutes before condensing	4	2085	satisfactory
15	Condensed skimmilk prepared from skimmilk which had been held at 85° C. for 30 minutes before condensing	2	2035	satisfactory
16	Homogenized superheated condensed (same as 13 but homogenized)	5	2025	satisfactory
18	Plain condensed skimmilk	1	1930	unsatisfactory
19	Homogenized plain condensed	1	1970	unsatisfactory

and had no appreciable effect on the baking quality. On homogenizing the regular superheated condensed skimmilk (No. 13), the viscosity decreased from 65° to 5° MacM. yet the baking quality was not impaired. The plain condensed skimmilk (No. 18) was also homogenized but the baking quality was not affected.

The baking procedure used in this work was of the sponge type, the milk being added at the dough stage. Sufficient milk was added in all cases so that 8% of skimmilk solids were used on the flour basis.

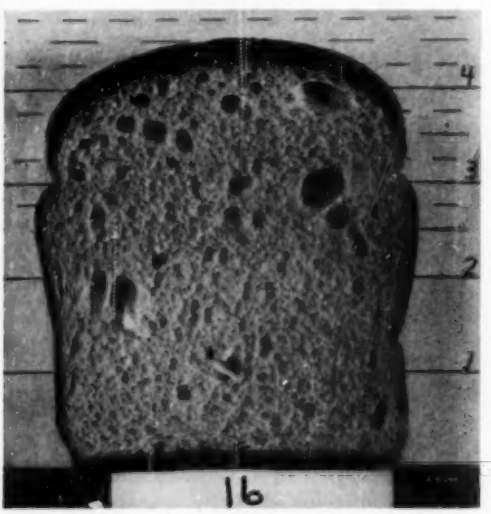
The baking data given in Table II show only loaf volume as the basis for determining baking quality. It should be mentioned that more important than loaf volume are other characteristics of the loaf. Some of these characteristics are revealed in Figure 1. Thus it is

shown that breads Nos. 18 and 19 have rather large, thick-walled cells with a texture that gives a dark color to the bread surface. Doughs Nos. 18 and 19 also had practically no oven spring which is apparent from the contour of the loaf. In addition, doughs Nos. 18 and 19 did not allow of as great absorption as did the adequately heat-

Regular superheated condensed skimmilk

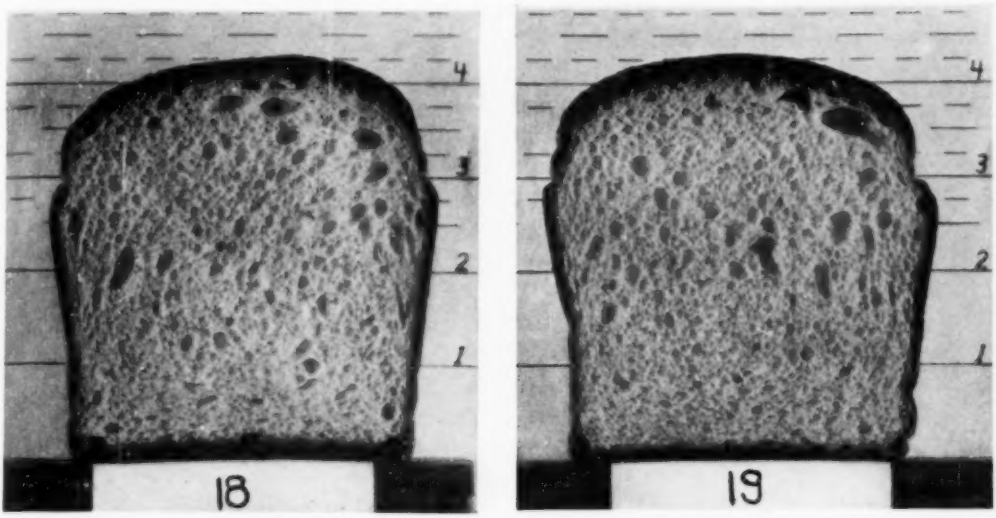
Superheated condensed skimmilk prepared from skimmilk which had been held at 85° C. for 30 minutes before condensing

Condensed skimmilk prepared from skimmilk which had been held at 85° C. for 30 minutes before condensing



Homogenized superheated condensed (same as 13 but homogenized)

Figure 1.—*Continued on opposite page.*



Non-superheated condensed skimmilk Homogenized plain condensed

Fig. 1. Photographs of bread baked from the various condensed skimmilks

treated milks included in this series, these latter milks allowing about 2% greater absorption.

It has now been shown that low viscosity is not necessarily an indication of poor baking quality in superheated condensed milks. The data in Table I actually show that some milks of low viscosity are superior in this respect to milks of higher viscosity. Thus sample No. 5837 of viscosity 64° MacM. was of unsatisfactory baking quality while sample No. 5719 of viscosity 39° MacM. was of satisfactory baking quality. Data are not available to show the heat treatments that were accorded these milks, but from our knowledge of the subject the likely explanation is that milk No. 5719 was accorded adequate heat treatment (but in such a way as not to result in high viscosity), while for milk sample No. 5837, the heat treatment was inadequate.

The Effect of Acidity on Heat Treatment Required to Increase the Viscosity

In order to produce condensed skimmilks of considerable viscosity but of poor baking quality, a series of experiments were conducted. It is known that old milks or slightly sour milks require much less heat treatment to cause increase in viscosity than do fresh milks of good quality. A plain condensed milk was accordingly condensed to 40% solids and dilute lactic acid and water added until the solids content was 30% and the acidity 1.24% as lactic acid. This acidified milk was then superheated by heating at 55° C. for the time required to

give the maximum viscosity. The superheating time at this temperature was about 2 minutes. Under these conditions the viscosity increased from about 1° MacM. for the plain condensed to 62° for the superheated. Such acidified milks are very difficult to superheat without "breaking" and even after many trials 62° was the highest viscosity that could be obtained without getting a milk which was of curdy texture.

Proper control milks were also included in this series as shown in Table III. These controls included a plain and superheated milk

TABLE III

VISCOSITY AND BAKING QUALITY OF ACIDIFIED PLAIN AND SUPERHEATED CONDENSED SKIMMILKS

Sample No.	Treatment	Viscosity at 27° C.	Loaf volume	General baking results
		° *MacM.*	*Cc.*	
1	Plain condensed skimmilk	1	1990	unsatisfactory
2	Plain condensed acidified to 1.24% and superheated for 2 minutes at 55° C., the maximum heat treatment that could be given without extreme coagulation	62	2010	unsatisfactory
3	Plain condensed acidified to 1.24% lactic acid	1	2015	unsatisfactory
4	Plain condensed acidified to 1.24% lactic acid, but the added acid neutralized before addition of the milk to the dough	1	2040	unsatisfactory
5	Superheated condensed skimmilk	68	2135	satisfactory
6	Superheated condensed acidified to 1.24% before addition of the milk to the dough	82	2120	satisfactory
7	Same as No. 6 but the added acid neutralized before the addition of the milk to the dough	50	2155	satisfactory

which had been acidified in the same way as the plain condensed and the plain and superheated milks in which the added acid was neutralized. The results in Table III show that these acidified milks or the milks neutralized after acidification were no different from the untreated milks in baking quality. It is clear moreover that the acidified milk which had been subjected to the maximum heat treatment that could be accorded it without causing curdling (*i.e.*, 2 minutes at 55° C.) and its viscosity thereby considerably increased,

was not improved in baking quality although the regular superheated milk of only slightly greater viscosity was of satisfactory baking quality. Thus, this milk apparently did not receive sufficient heat treatment to render it satisfactory for baking.

The breads produced from milks Nos. 1, 2, 3, and 4 were much like bread No. 18 shown in Figure 1, while those produced from Nos. 5, 6, and 7 were like No. 13. The unsatisfactory breads were not only lower in loaf volume but the texture was inferior, the optimum absorption about 2% lower, and in baking, the doughs showed little or no oven spring.

It is recognized that an acidity of 1.24% (as lactic) is very high and resulted from increasing by 0.70% the acidity of the original condensed milk. An experiment was therefore conducted in which the milk was allowed naturally to sour slightly before proceeding with the superheating operation. The finished milk in this case had an acidity of 0.63%. A heat treatment of this milk for 5½ minutes at 70° C. yielded a superheated milk of viscosity 59° MacM. This was about the maximum heat treatment that could be accorded this milk without causing "breaking." The bread produced from this product was unsatisfactory showing the characteristic lower loaf volume, coarse texture, and lack of oven spring.

The Effect of Freezing on Viscosity and on Baking Quality

There are methods other than the application of heat by which the viscosity of condensed skimmilks may be increased. It is well known that on freezing the heat stability of milks is greatly reduced. Thus, Webb and Hall (1935) have shown that if the conditions as to solids content and freezing temperature are properly controlled, milks of varying heat stability can be obtained or even milks of very high viscosity without the application of any heat (therefore milks of zero heat stability). Webb and Hall have shown that the high solids content of condensed milk and the higher freezing temperatures operate rapidly to change the body of the milk during storage. Since the freezing of plain condensed milk offered a means of markedly increasing the viscosity of the milk without subjecting it to any heat treatment, it was thought of interest to freeze and hold two series of condensed skimmilks, one at − 10° C. and the other at − 18° C. and determine the effects of such freezing on the baking quality. These milks were prepared on March 18, 1936, and placed in frozen storage one day later. On April 1, and on May 12, samples of each milk were removed from cold storage, thawed by allowing to remain at room temperature overnight and subjected to baking tests and viscosity tests as far as it was possible to make the latter. The results of the viscosity tests are

given in Table IV. These data confirm the results of Webb and Hall that higher freezing temperatures operate to allow rapid increase in viscosity of the condensed product and that high or long heat treatment reduces the heat stability or allows greater increase in viscosity during a given storage period.

TABLE IV

THE VISCOSITY OF VARIOUS CONDENSED SKIMMILKS THAT HAVE BEEN HELD IN FROZEN STORAGE

Sample No.	Description of milk	Original viscosity March 18	Viscosity on Milks Frozen until April 1		Viscosity on Milks Frozen until May 12	
			at −10° C.	at −18° C.	at −10° C.	at −18° C.
		° *MacM.*	° *MacM.*	° *MacM.*	° *MacM.*	° *MacM.*
13	Superheated condensed, superheated at 85° C. for 17 minutes	65	160	115	Lumpy and gelatinous	275
14	Same as preceding except preheated at 85° C. for 30 minutes before condensing and superheating	4	182	38	Lumpy and gelatinous	47
15	Plain condensed preheated to 85° C. for 30 minutes before condensing	2	115	4	Lumpy and gelatinous	10
16	Homogenized superheated condensed	5	—	—	Lumpy and gelatinous	18
18	Regular plain condensed preheated to 68.5° before condensing	1	32	1	Lumpy and gelatinous	2
17	Plain condensed run directly into the vacuum pan with no preheating	1	18	1	Lumpy and gelatinous	3
19	Homogenized plain condensed	1	—	—	Lumpy and gelatinous	2

In the case of the milks listed as having viscosities too great to run, the products were in many cases lumpy and gelatinous in nature and the bob of the viscosimeter would merely revolve in the gelatinous mass being lubricated by liquid which separated from the mass.

The thawed condensed milks listed in Table IV were also subjected to baking tests. Wherever adequate heat treatment was accorded the milk, satisfactory baking results were obtained. Thus the bread prepared from milks Nos. 13, 14, 15, and 16 was satisfactory regardless of the viscosity or condition of the milk after thawing. On the other

hand, breads prepared with milks Nos. 17, 18, or 19 were always unsatisfactory.

It would appear, therefore, that the changes in properties of condensed skimmilk effected by heating and by freezing are quite different as far as bread-baking properties are concerned. Both of these treatments operate to increase the viscosity of the product which effect is probably due to a change in the properties of the casein. This would indicate that viscosity is not the deciding factor in determining whether a condensed milk is suitable for breadmaking. In many cases, however, it may serve to indicate whether the milk has been properly heat treated.

Summary

The viscosity of superheated condensed skimmilk is not necessarily an indication of baking quality.

Heat treatment appears to determine how a condensed milk will react in breadmaking.

Adequate heat treatment of the milk is necessary in order that the milk allow proper absorption in the dough and that the bread show satisfactory texture, loaf volume, and oven spring. This heat treatment may or may not be accorded the milk in such a way as to increase the viscosity of the superheated product.

Increase in acidity of the milk either naturally or by the direct addition of lactic acid allowed the development of considerable viscosity with a minimum of heat treatment, yet the baking results with viscous, acidified, heat-treated milk were unsatisfactory.

Freezing of plain condensed skimmilk and holding the frozen milk in storage yielded milks of high viscosity yet baking results with such milks were unsatisfactory.

Freezing of heat-treated condensed skimmilk and holding the frozen milks in storage yielded milks of high viscosity and the baking results were satisfactory.

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EFFECT OF MIXING ON THE PHYSICAL PROPERTIES OF DOUGH[1]

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Mixing Studies.

The objective of dough mixing is to uniformly incorporate the ingredients and to hydrate the gluten and starch. Markley, Harrington, and Bailey (1935) have shown that low protein flours tend to develop more quickly, as measured by the farinograph, than high protein flours, as evidenced by the time required to reach the highest value on the farinograph curve.

The type of mixer used and the speed of the rotating arms influence the time of mixing required for optimum development. It is well known in commercial practice that the time necessary to mix the dough is related to the speed of the mixing arms. Thus, a slow speed, two barrel mixer, revolving at 30 r.p.m., was found to require 22 minutes for complete mixing, whereas a high speed, two barrel mixer, revolving at 60 r.p.m., and with somewhat similar mixing arms, only required from 8 to 10 minutes for complete mixing of the same flour dough. Very high speed mixers, revolving at 80 r.p.m., require about 6 to 8 minutes. It is claimed that high speed mixers make it possible to incorporate slightly larger amounts of water into the dough than low speed mixers. The increase does not appear to be very significant, providing each dough is mixed to approximately the same stage of development.

The baker determines the optimum mixing time by the feel of the dough. In the first stages of mixing, the flour, water and other ingredients are very unevenly distributed. As mixing proceeds the dough gradually becomes smooth and forms a continuous mass; it soon appears to "dry up," as the baker terms it. If mixing is continued past this stage the dough tends to slacken and become sticky. The skilled baker can judge this optimum point of mixing time with considerable accuracy. Commercial tests carried on at the Canadian School of Baking have indicated that the time of mixing which gives maximum dryness of the dough results in the optimum loaf of bread.

The maximum reading on the farinograph curve appears to be the

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time of optimum dough development as far as baking quality is concerned. It was thought advisable to check this assumption on samples of strong and medium strength flours (Tables I and II). Tests were randomized and conducted in duplicate. The formula used was as follows:

Formula 2 (Farinograph)

Flour	300 g.	Temperature dough	30° C.
Water. Mixed to 540 farinograph units		Time 1st punch	110 min.
Yeast	3%	Time 2nd punch	50 "
Salt	2%	Time pan	25 "
Sugar	3½%	Proofed to definite height at	30° C.
Shortening	2%	Baked at	227° C.

TABLE I

EFFECT OF DIFFERENT MIXING TIMES ON THE BAKING QUALITY OF STRONG FLOUR DOUGH

Time of mixing in farinograph	Farinograph reading	Loaf volume	Color	Texture	Grain	Loaf type	Baking value
Minutes	*Units*	*Cc.*	*Score*	*Score*	*Score*		*Score*
10.0	500	553	97.5	98.0	97.5	9.0	65.3
15.0	550	548	98.0	98.5	97.0	9.0	67.8
22.5	535	515	97.5	97.5	96.0	8.5	61.0
30.0	520	488	96.0	95.0	95.0	8.0	51.8
3.0[1]	—	593	98.5	99.0	97.0	9.0	73.3

[1] Mixed in Hobart-Swanson machine.

TABLE II

EFFECT OF DIFFERENT TIMES OF MIXING ON THE BAKING QUALITY OF MEDIUM STRENGTH FLOUR DOUGH

Time of mixing in farinograph	Farinograph reading	Loaf volume	Color	Texture	Grain	Baking type	Baking value
Minutes	*Units*	*Cc.*	*Score*	*Score*	*Score*		*Score*
5	500	508	99.0	98.0	97.5	7.5	61.3
10	560	525	99.0	99.0	98.0	8.0	65.5
15	545	510	99.0	98.0	97.5	8.0	62.0
20	525	488	98.0	96.0	97.0	7.5	56.3
25	500	480	98.0	95.5	96.5	7.0	53.5
2[1]	—	540	99.5	99.5	98.0	8.5	68.0

[1] Mixed in Hobart-Swanson machine.

The 15-minute mixing of strong flour dough and 10 minute mixing of medium strength flour dough gave the best baking results. This corresponds to the maximum reading on the farinograph.

Baking tests were also conducted with doughs mixed for varying periods in the Hobart-Swanson machine. It was found that the 3

minutes mixing of strong flour dough and 2 minutes mixing of medium strength flour dough gave the optimum baking results according to the standard A.A.C.C. baking test with 2% shortening and a constant proofing height. The optimum mixing time of a dough with a baking mixture (Formula 2) should not be confused with a flour-salt-water dough (Formula 1). The latter requires an appreciably shorter mixing time.

Formula 1

Flour	300 g.
Water	Mixed to 540 farinograph units
Salt	2%

It will be noted from Tables I and II that the doughs mixed in the Hobart-Swanson mixer gave a loaf of higher baking value than those mixed by the farinograph. The former represents an extremely high speed mixer, whereas the farinograph is a slow speed mixer. It has also been observed in the case of a few tests that the stress readings run on the stress meter described by Bohn and Bailey (1936) were somewhat higher in the case of the doughs prepared by the Hobart-Swanson mixer than in doughs mixed in the farinograph. The same absorption was used in both mixers and the doughs were mixed to the optimum time of mixing. Doughs mixed in the farinograph required about 1% less absorption to give the same consistency or feel to the hand, however.

Since mixing time has been shown to influence the bread baking characteristics of dough, a study was made of the effect of different periods of mixing on the stress meter readings. The first experiments were carried out on a simple flour, water and salt dough. Tables III and IV show the results of such readings of doughs prepared in the farinograph mixer.

TABLE III

THE EFFECT OF MIXING TIME ON THE STRESS READINGS (STRONG FLOUR-FARINOGRAPH AT 30° C.)

Time of mixing in farinograph	Height farinograph curve	Stress readings			
		15 Sec.	30 Sec.	1 Min.	2 Min.
Minutes	*Units*	*Grams*	*Grams*	*Grams*	*Grams*
3	500	16.4	12.7	10.3	8.1
5	520	16.5	13.4	10.9	8.8
10	555	16.0	12.7	10.8	8.6
15	530	10.7	8.4	7.0	5.7
20	500	9.3	6.9	5.5	4.3
25	445	6.4	4.8	3.8	2.9
30	405	3.2	2.1	1.8	1.4

TABLE IV

THE EFFECT OF MIXING TIME ON THE STRESS READINGS (MEDIUM STRENGTH FLOUR—USING THE FARINOGRAPH AT 30° C. FOR MIXING)

Time of mixing in farinograph	Height farinograph curve	Stress readings			
		15 Sec.	30 Sec.	1 Min.	2 Min.
Minutes	*Units*	*Grams*	*Grams*	*Grams*	*Grams*
2.5	515	13.5	10.7	8.7	6.7
5	550	12.4	10.0	8.2	6.4
7	570	—	—	—	—
10	555	10.5	8.0	6.3	5.0
15	540	7.7	6.0	5.1	3.9
20	510	6.0	4.5	3.5	2.9
25	490	4.8	3.7	3.0	2.2
30	455	3.9	3.1	2.4	2.0

Unless otherwise stated, the stress readings reported hereafter are the average of the readings taken 10, 15, and 20 minutes after the dough comes from the mixer. In the majority of dough mixing experiments which follow, the mixing time was varied, but the time interval which elapses between the end of mixing and the time of taking stress readings was kept constant. Stress readings were taken 15 seconds, 30 seconds, 1 minute, and 2 minutes after commencing to elongate the dough on the stress meter. The time required to elongate the dough to five times the original length of the test piece was 10 seconds.

Using a flour, salt and water mixture there is seen to be no significant difference in the stress readings of the 3, 5 and 10 minute mixing treatments of the strong flour as shown in Table III. The 10 minute farinograph reading was the highest for the strong flour.

In the case of medium strength flour (Table IV) the 2.5 and 5 minute mixing treatments gave approximately the same stress readings, whereas the 7 minute mix gave the highest farinograph reading of 570 units.

After 10 minutes mixing of the strong flour and 7 minutes mixing of the medium strength flour, the stress readings fell off rapidly. In fact, the reduction in stress reading values, due to the so-called "breaking down" of the gluten, is seen to be much more rapid than the reduction in farinograph values.

It was somewhat surprising to note that the stress values with short periods of mixing were about the same as those at the optimum time of mixing. It should be kept in mind, however, that the dough has an average rest of 15 minutes before the stress readings are taken and that during this time the gluten has an opportunity to become more uniformly hydrated.

A series of stress determinations was next made on strong and medium strength flours mixed for different times in a Hobart-Swanson mixer (Tables V and VI), using a simple flour-water-salt mixture.

TABLE V

THE EFFECT OF MIXING TIME ON THE STRESS READINGS (STRONG FLOUR—USING THE HOBART-SWANSON MIXER—TEMP. 30° C.)

Mixing time Hobart-Swanson machine	Stress readings			
	15 Sec.	30 Sec.	1 Min.	2 Min.
Minutes	*Grams*	*Grams*	*Grams*	*Grams*
1	18.3	14.7	11.8	9.3
2	21.7	17.1	13.9	11.1
3	16.0	12.8	10.6	8.6
4	13.5	9.8	Broke	Broke
5	12.6	9.3	Broke	Broke

TABLE VI

THE EFFECT OF MIXING TIME ON THE STRESS READINGS (MEDIUM STRENGTH FLOUR—USING THE HOBART-SWANSON MIXER—TEMP. 30° C.)

Mixing time Hobart-Swanson machine	Stress readings			
	15 Sec.	30 Sec.	1 Min.	2 Min.
Minutes	*Grams*	*Grams*	*Grams*	*Grams*
1.0	17.0	13.0	10.9	8.3
1.5	17.1	13.8	11.4	9.0
2.0	17.4	13.8	10.9	8.8
3.0	12.3	9.7	8.2	6.7
4.0	Broke	—	—	—

Of the Hobart-Swanson mixing treatments studied, the 2 minute mixing time for the strong flour and the 1½ minute mixing for medium strength flour gave the highest stress readings. Both the strong flour and the medium strength flour fell off rapidly in stress values after the optimum time of mixing was exceeded. It is usually found that five minutes mixing in the Brabender farinograph is equal to about one minute mixing in the Hobart-Swanson mixer.

The dough formula used has a marked effect on the time required to reach the optimum development of the dough. Salt, milk, and shortening have the most pronounced effects upon the time required to reach optimum development, as indicated by the highest point on the farinograph curve, whereas yeast, sugar, and malt have a lesser effect.

The results of varying the mixing time in the farinograph on the stress readings in grams per square centimeter, using a baking formula (Formula 2 + 1% malt flour), are recorded in Table VII and graphed in Figure 1. Where the stress readings are corrected for area, the

TABLE VII

THE EFFECT OF DIFFERENT MIXING TIMES ON THE STRESS READINGS IN GRAMS PER SQUARE CENTIMETER (STRONG FLOUR—USING THE FARINOGRAPH AND FORMULA 2 + 1% MALT FLOUR—26° C.)

Time of mixing in farinograph	Stress readings				Area-test piece	Farinograph readings
	15 Sec.	30 Sec.	1 Min.	2 Min.		
Minutes	*g./cm.²*	*g./cm.²*	*g./cm.²*	*g./cm.²*	*cm.*	*Units*
6	11.3	8.6	7.2	5.6	1.23	440
10	14.1	10.8	8.8	7.3	1.32	500
15	17.3	13.0	10.4	8.8	1.02	550
20	14.3	11.1	9.1	7.3	1.13	540
30	10.8	7.9	6.5	5.1	1.21	520

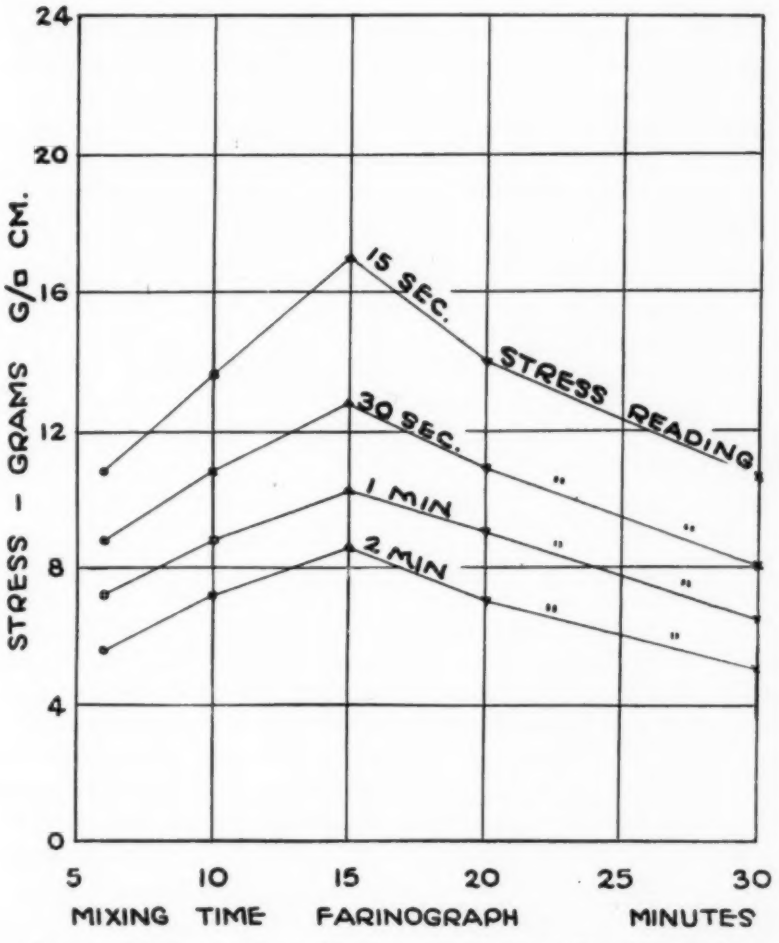

Figure 1. The effect of different mixing times upon the stress readings of flour doughs.

highest readings were obtained at the end of 15 minutes mixing and they fell off appreciably on each side of this point. Differences in the farinograph readings are not very great between doughs mixed for 10 and 30 minutes, however.

A significant fact brought out by the stress readings is that after the maximum point on the farinograph is reached the stress readings as given by the stress meter decrease considerably, indicating that marked changes are taking place in the condition of the gluten during prolonged mixing, even though this may not be so evident from the farinograph readings and from biochemical studies as carried out by Skovholt and Bailey (1935).

The Effect of Time of Rest of Dough on Stress Readings

Before arriving at an established technique for determining stress on the stress meter described by Bohn and Bailey (1936) and in which the stress value is taken as the average of the stress readings 10, 15, and 20 minutes after the end of mixing, stress tests were run at the end of 5, 10, 15, 20, 25, and 30 minutes after the end of mixing. These preliminary tests were made on strong, medium strength and cake flours. Twenty-four of these sets of 15-second stress readings were averaged with the results recorded in Table VIII.

TABLE VIII

THE EFFECT OF DIFFERENT REST PERIODS AFTER MIXING AND PRIOR TO TAKING STRESS READINGS (AVERAGE OF 24 DETERMINATIONS)

Time of rest after mixing before taking stress readings, Minutes	5	10	15	20	25	30
Average of 15-second stress readings, Grams	9.76	13.19	13.95	14.20	13.93	13.89

Using the conception of the protein molecule and micelle advanced by Dorothy Jordan Lloyd, it is conceivable, as pointed out by Skovholt and Bailey (1935), that during mixing the gluten molecules are elastic, but are being pulled past each other due to the plastic flow which is taking place. The amount of plastic flow due to the proteins would be dependent upon the forces of attraction between the hydrated protein molecules and/or the resistance to flow of one protein molecule along others. It is possible that with the increase in broken linkages between protein molecules during mixing there may be a decrease in resistance offered to plastic flow by the protein molecules which again increases after the dough is allowed to stand after mixing.

The stress readings obtained on the stress meter are a measure of plastic or mobile flow, even though the shape of the test piece is not changing during the test period. Moreover, the 5-minute rest period before taking stress readings is always found to give decidedly low results. The 10-minute rest period readings are also a little lower than the 15-minute rest period readings as a general rule. The latter differences were not great, however, and so the average of the 10, 15, and 20 minute rest periods was chosen in standardizing the technique for stress meter readings.

It is known that certain overmixed doughs which are very slack on coming out of the mixer tighten up considerably on standing. It is possible that if stress readings were measured in doughs without yeast or added enzyme preparations, an increase in stress readings would be observed during the first few minutes after mixing.

Overmixing cannot be substituted for fermentation. Swanson and Working (1926) demonstrated that mechanical action on dough may be made to take the place of the usual fermentation prior to panning.

In the light of the data in the present study, a slightly different interpretation might be placed upon the findings of Swanson and Working. As previously shown, both fermentation and excessive mixing (*i.e.*, a mixing time beyond the dry stage to the baker or the maximum reading on the farinograph) mellow or soften the gluten in such a manner as to decrease the stress readings. On the other hand, high speed commercial mixers as well as the Hobart-Swanson mixer appear to increase the elasticity of the dough, somewhat analogous to an increase in strength of flour, as evidenced by the fact that the stress readings are higher than where slow speed mixers or the Brabender farinograph are used (compare Tables III and IV with V and VI).

It has frequently been noted in commercial practice that high speed mixers will give doughs that feel slightly more elastic and will absorb slightly more water than slow speed mixers. Moreover, high speed mixers will usually give very slightly better baking results, with strong flours at least, than slow speed mixers, assuming both are mixed to the dry stage or to the optimum stage of development. It should be kept in mind that a slow speed mixer requires a much longer time to reach an optimum stage of development than a high speed mixer. If a dough in the slow speed mixer is undermixed, whereas the dough in the high speed mixer is mixed to the dry stage, the difference in the loaves baked therefrom would be expected to be distinctly in favor of the high speed mixer. It has been observed in commercial experience that the difference between the bread produced by high and low speed mixers will not be very great with strong flours providing each dough is mixed to its optimum value.

Moreover, overmixing a dough, beyond the dry point or optimum value, as indicated by the maximum reading on the farinograph curve, cannot be substituted for fermentation. Overmixing in an attempt to break down the gluten, injures the baking value regardless of how short the subsequent fermentation may be. It is recognized that both fermentation and overmixing lower the stress values, but their effects are different on the finished loaf of bread. Data are presented in Tables IX and X, to bear out this contention.

TABLE IX

THE EFFECT OF DIFFERENT MIXING TREATMENTS ON THE BAKING QUALITY OF BREAD NOT GIVEN REGULAR FERMENTATION PERIOD (SO-CALLED NO TIME DOUGH)—(MEDIUM STRENGTH FLOUR—FARINOGRAPH MIXER—TEMPERATURE OF DOUGH 30° C.)

Loaf	Time of mixing in farinograph (30° C.)	Time to 1st punch	Time to 2nd punch	Time to pan	Total mixing and fermentation time	Loaf volume
Number	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Cc.*
1	10	110	50	25	195	575
2	10	—	—	70	80	650
3	20	—	—	60	80	645
4	30	—	—	50	80	645

TABLE X

BREAD SCORE (EXPERIMENTS DESCRIBED IN TABLE IX)

		Loaf number			
	Score	1	2	3	4
External Characteristics					
Volume	10	9	10	10	10
Color of crust	8	7.5	7.5	7.5	7.5
Symmetry of form	3	2.5	2.0	1.5	1.5
Evenness of bake	3	2.5	2.5	2.5	2.5
Break or shred	3	2.5	1.5	1.0	1.0
Character of crust	3	2.5	2.5	2.5	2.5
	30	26.5	26.0	25.0	25.0
Internal Characteristics					
Color of crumb	10	9.0	9.5	9.5	9.5
Texture or feel	15	13.5	14.0	13.5	13.5
Grain	10	9.0	9.0	9.0	8.0
Taste	20	18.5	17.0	16.5	16.0
Smell	15	14.5	14.0	14.0	14.0
	70	64.5	63.5	62.5	60.0
	100	91.0	89.5	87.5	85.0

The loaves obtained from the schedule recorded in Table IX were duplicated and randomized. They were judged according to the Official Score of the American Bakers Association, since all bread

characteristics, and especially taste and smell, should be considered. The bread was commented upon independently by four different workers. The bread scores recorded in Table X were assigned to the loaves. Loaves 2, 3, and 4 were just given enough fermentation to facilitate panning. The carbon dioxide first formed is dissolved in the dough which accounts for the long panning time of loaves 2, 3, and 4 as compared with loaf No. 1, which was given a standard A.A.C.C. fermentation time. Loaves 2, 3, and 4 were given a total mixing time and time to pan of 80 minutes. Loaves were proofed to a constant height at 30° C.

The volumes of the loaves 2, 3, and 4, so-called "no time doughs," were greater than the fermented dough and they were also slightly whiter in color of crumb, and somewhat darker in crust color (due to a higher sugar content) than loaf No. 1. On the other hand, loaves No. 2, 3, and 4 were inferior to No. 1 in symmetry of form, break or shred, and taste and smell. The unfermented doughs (Nos. 2, 3, and 4) had wild breaks and uneven symmetry. Moreover, they had a sweetish taste as compared with a fermented taste in the fermented dough. The fermented taste is usually accepted as the better bread taste.

Loaf No. 2 was superior to loaves 3 and 4 in symmetry of form, break or shred, texture or feel, and taste (*i.e.*, better feel of the bread while chewing, rather than taste proper which only records sweet, sour, bitter and salt).

The results would indicate that a properly fermented dough yielded bread superior to that baked from an unfermented dough and that a dough mixed to a maximum point on the farinograph curve gave better baking results than a dough which was overmixed. Similar results were obtained on strong flour, using a 15 minute optimum mixing time in this case. The evidence, limited as it is (though borne out by unreported commercial tests), tends to bear out the contention that overmixing, in itself at least, cannot be substituted for fermentation. Where so-called "no time" doughs are desired, increasing the temperature of mix to 90–95° F., and increasing the enzymic activity of the dough might be resorted to advantageously.

Effect of Temperature on Physical Properties of Doughs

Skovholt and Bailey (1932) have shown that the height of the farinograph curve varied markedly with temperature. Another point of interest that does not appear to have been previously reported is that the time required to reach an optimum value, as shown for strong and medium strength flours, is the same regardless of the temperature. This would indicate that temperature has no effect on the time of optimum mixing or dry point in dough development. This appears to

be borne out in commercial practice, since no adjustment is ordinarily made in mixing time for changes in mixing temperature.

Determinations were made to find the effect of temperature on the stress readings. Doughs were mixed in the farinograph at 20, 25, 30, and 35° C. and were held at that temperature while making stress readings. The dough gun and stress meter were held at the temperature of the experiment. The results of these tests are shown in Table XI and Figure 2. A strong flour was used and the doughs were mixed for 15 minutes in the farinograph (*i.e.*, the maximum value) using Formula 2. The farinograph readings changed at an average rate of 18.7 farinograph units per degree Centigrade change in temperature. The amount of change per degree Centigrade appears to be fairly uniform in the case of the farinograph between 20° and 35° C.

TABLE XI

EFFECT OF TEMPERATURE ON STRESS READINGS (STRONG FLOUR DOUGH—STRESS TESTS CORRECTED FOR AREA OF TEST PIECE)

Temperature	Maximum farinograph reading	Area test piece	Stress readings			
			15 Sec.	30 Sec.	1 Min.	2 Min.
° C.	*Units*	*cm.²*	*g./cm.²*	*g./cm.²*	*g./cm.²*	*g./cm.²*
20	665	0.95	25.4	19.3	15.8	13.2
25	575	1.15	18.7	13.6	11.2	9.5
30	475	1.39	10.5	8.3	6.8	5.8
35	385	1.54	6.9	5.4	4.2	3.4

The changes in the stress readings with change of temperature are seen to be extremely large. The decrease in 15 second stress readings per degree Centigrade increase in temperature varied from about 1.3 to 2 grams per square centimeter as the temperature was varied from 35° C. to 20° C. The average decrease in stress readings per degree Centigrade increase in temperature was 4.9%, whereas the farinograph readings showed a decrease of 2.8%.

Large differences in temperature seriously affect the accuracy of stress readings (or farinograph readings for that matter) and the temperature must be accurately controlled. It so happens, however, that the chief variation in stress readings is due to variations in the temperature of the dough during mixing. If the temperature of the dough is held constant during mixing, variations in temperature at the time of taking stress meter readings are much smaller than those reported in Table XI.

Effect of Absorption on Physical Properties of Doughs

The quantity of water used in mixing a dough has a decided effect upon the consistency, and it would be expected, therefore, to appreci-

ably affect both farinograph and stress meter readings. As the absorption is increased the height of the farinograph curve decreases and the dough becomes more mobile. The time required to reach the maxi-

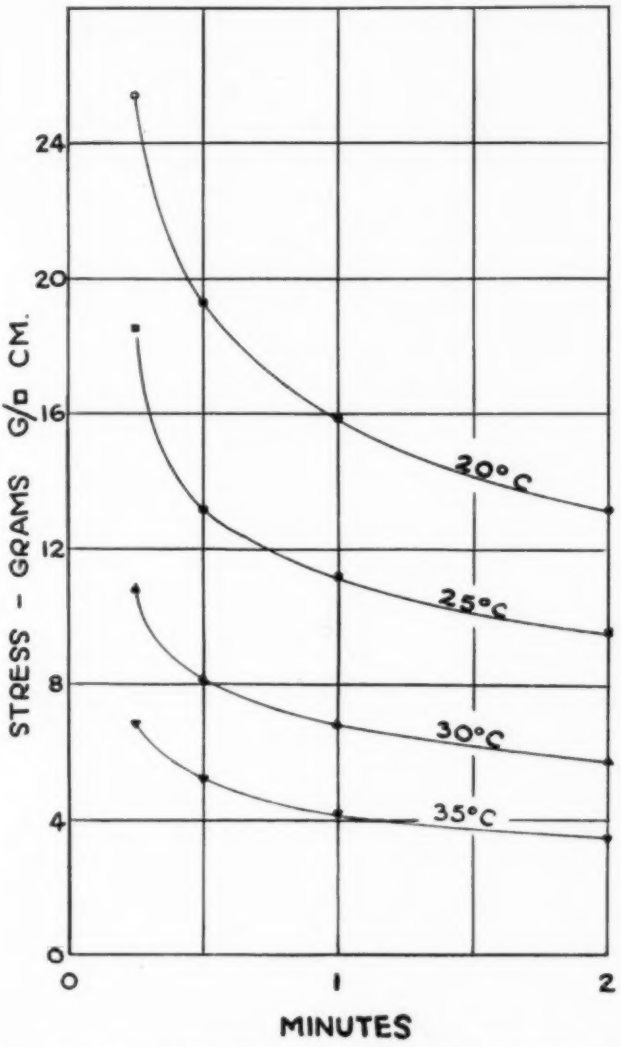

Figure 2. The effect of different temperatures of the dough during mixing upon the stress readings of flour doughs.

mum point on the farinograph curve increases as the absorption is increased. Since more work is put into a stiff dough per unit of time in the mixing process than a slack dough, it would appear that this

affects the time of optimum development. Tables XII and XIII, and Figures 3 and 4, show the effect of different proportions of water on the stress readings. A simple flour, water and salt dough was used.

TABLE XII

EFFECT OF DIFFERENT ABSORPTIONS ON STRESS AND FARINOGRAPH READINGS (FLOUR, WATER AND SALT DOUGH—STRONG FLOUR)

Water used	Time to maximum point on farinograph	Height to maximum point on farinograph	Stress readings			
			15 Sec.	30 Sec.	1 Min.	2 Min.
%	*Minutes*	*Units*	*Grams*	*Grams*	*Grams*	*Grams*
62	13.5	590	25.1	18.3	15.1	11.9
65	15.0	535	21.9	16.4	13.1	10.0
68	19.0	485	18.2	14.2	11.3	8.9
71	23.0	445	14.6	11.7	9.0	7.3

TABLE XIII

EFFECT OF DIFFERENT ABSORPTIONS ON STRESS AND FARINOGRAPH READINGS (FLOUR, WATER AND SALT DOUGH—MEDIUM STRENGTH FLOUR)

Water used	Stress readings			
	15 Sec.	30 Sec.	1 Min.	2 Min.
%	*Grams*	*Grams*	*Grams*	*Grams*
61	17.5	13.5	10.8	8.7
64	15.4	12.3	9.3	7.4
67	13.7	10.4	8.5	6.6
70	11.0	8.4	6.8	5.5

The maximum farinograph reading was found to decrease approximately 16 units with each increase of one per cent in water used. The 15 second stress readings were found to decrease about 1.2 g. in the case of strong flour and about 0.7 g. in the case of medium strength flour for a one per cent increase in absorption. The other stress readings show about the same percentage decrease. It is thus evident that the consistency or absorption of the dough must also be carefully controlled as well as the temperature, if dependable stress readings are to be obtained on different flours. One of the greatest obstacles to accurate results in mechanical methods of testing the physical properties of flours seems to have been the inability to control the consistency of the dough. Some type of plasticity-measuring device such as the farinograph, makes possible a fairly accurate control of dough consistency. The farinograph also facilitates control of the temperature of the dough during mixing.

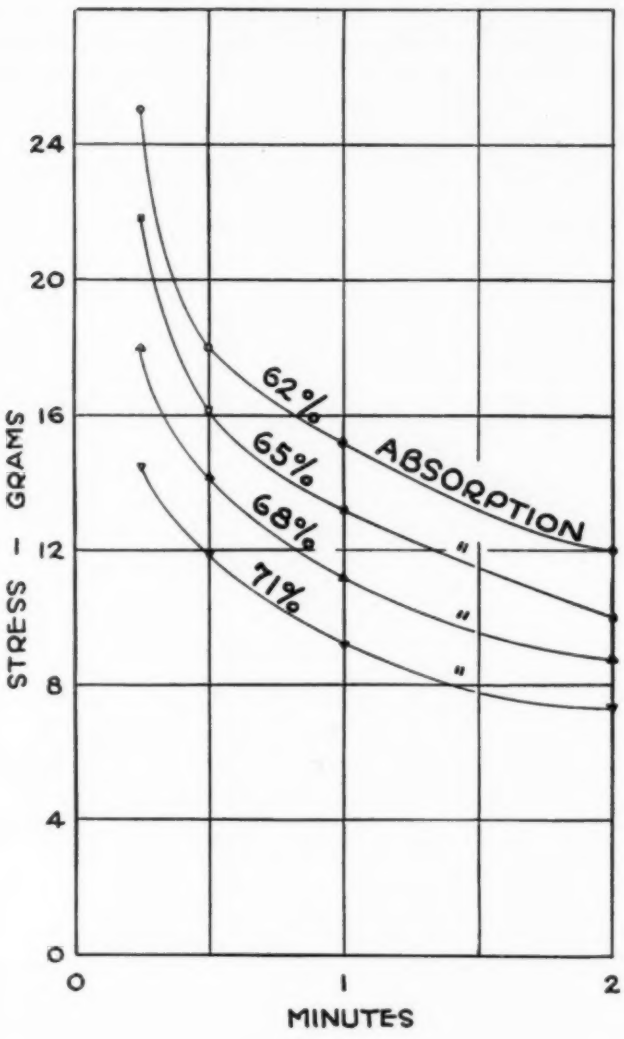

Fig. 3.

Summary

Excessive mixing of a dough beyond the point of optimum development tends to produce an inferior loaf of bread in volume, texture, grain, and eating properties.

High speed mixers develop a dough more quickly and tend to give higher maximum stress values than low speed mixers. The high speed Hobart-Swanson mixer also necessitated the use of slightly more water in the dough and gave a slightly better loaf than the Brabender farinograph low speed mixer.

Excessive mixing cannot be substituted for fermentation. Regardless of the length of fermentation, doughs mixed to the maximum peak on the farinograph curve or the dry stage in dough development, give the best baking results in bread.

Stress readings tend to fall off markedly after the point of optimum dough development is reached, even though this is not so evident from

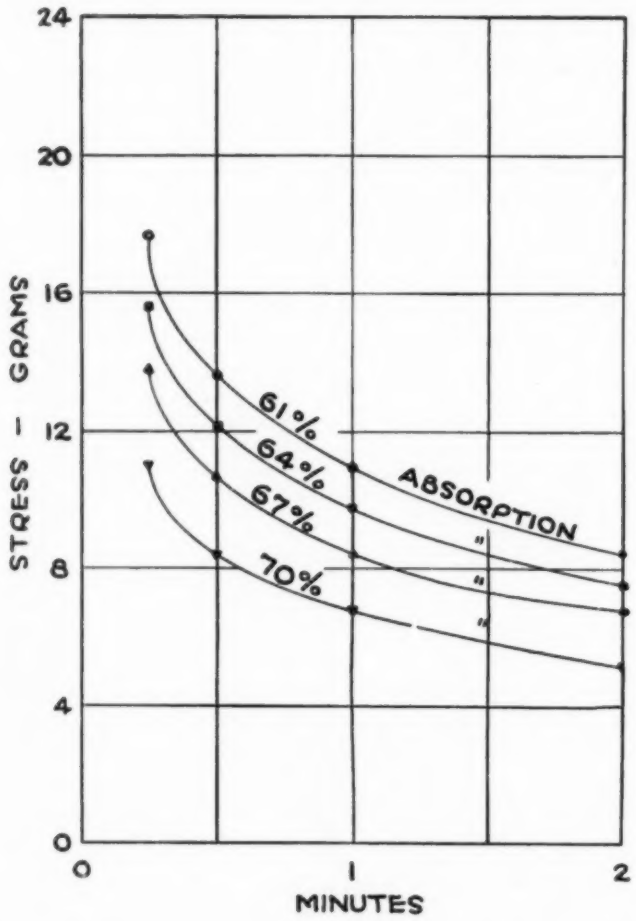

Fig. 4.

Figures 3 and 4. The effect of different absorptions upon the stress readings of doughs.

farinograph values. This indicates that overmixing produces marked physical changes in the plastic properties of dough.

Dough formula used affects the time required to reach optimum dough development. Doughs mixed between 20° and 35° C. had the same optimum mixing time. Increasing the water used increased the required time of mixing.

Doughs allowed to rest for 15 minutes after coming from the mixer gave higher stress readings than freshly mixed doughs, indicating that a freshly mixed dough is in an unstable physical state.

Increasing the temperature of dough mixing from 20° to 35° C. decreased the stress readings of doughs to less than a third of their original value.

Increased percentages of water in doughs decreased the stress readings.

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OBSERVATIONS ON GLUTEN WASHING[1]

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Introduction and Review of Some Earlier Work

The importance of the rôle of the flour proteins in the production of good bread was realised many years ago, and in consequence, the washing-out of gluten became a popular flour test; for not only does the amount of gluten obtained give a fair measure of the protein content of the flour but in the hands of an experienced operator the feel of the gluten gives some indication of its quality. The test, however, has not during recent years been regarded with so much favour, probably because of the difficulty of obtaining exact replication due to the dominating influence of personal errors. Many attempts have been made to bring it more into line with exact chemical tests, to make it, in fact, a determination of precision, and since, at the present time, methods are being evolved in an endeavour to measure factors contributing to gluten quality, this question of washing-out gluten has assumed a new importance.

[1] Issued for private circulation in 1932 as Confidential Report No. 22 of the Research Association of British Flour Millers. Released for publication by the Council of the Research Association, 1935.

As a preliminary to work on the quality aspect of gluten it was considered desirable to investigate the various methods of gluten washing as practised in different laboratories.

In a manual, by Marcel Arpin, published in 1913, under the auspices of the French Government for use in its official laboratories, the author draws attention to " this estimation, so simple to make in appearance and so difficult however in performance if one wishes to obtain from the same sample results which only differ from one another insensibly." He goes on to state that among the causes which influence this estimation and which are capable of making considerable variations in the results are the following: (1) the composition of the water used in washing-out, (2) the temperature of this water, (3) the length of resting time of the pellet of dough before extraction, and (4) the duration of the extraction.

Dealing with the first of these he states that distilled water has a solvent action on gluten but that this action is arrested when the water contains sufficient saline matter. It is, however, he continued, of primary importance to choose a water that is not too heavily charged with salts as salt solutions, although not affecting the quantity of gluten washed-out, augment somewhat its water-absorbing power. For the official French method he recommends water containing about 100 mg. of total lime per litre, of which 80 to 90 mg. should be in the form of carbonate.

Arpin found that when the temperature of the washing water was raised the quantity of gluten obtained was increased, at the same time the water absorption of the gluten being slightly increased. For example, in one case increasing the temperature of the wash water from 5° to 35° C. resulted in the amount of gluten obtained being increased from 7.83% to 8.52%, the water absorption at the same time being only slightly increased, from 67.2% to 68.3%. He advises a temperature of about 16° C.

Stronger flours of higher gluten content than the French flours studied by Arpin, appear to be less sensitive to the temperature of washing water. Thus, with one flour the writers found the following dry gluten contents at 11° C. and 18° C., respectively: 13.3, 13.2, 13.0, 13.3, 13.2, 13.2—mean 13.2% at 11° C., and 13.8, 13.3, 13.4, 13.3, 13.1, 13.2—mean 13.35% at 18° C. Differences in daily laboratory temperature would not be expected to cause significant differences in gluten determination.

Arpin recommends 33.33 g. of flour for making the dough, an operation that takes about 3 minutes. He then washes the dough under a stream of water for about 7 minutes, stopping when the water is no longer white but only slightly turbid. He states that it is necessary to

avoid prolonging the washing beyond this point so as not to dissolve any gluten.

In 1924, Dill and Alsberg emphasized the importance of the composition of the water used in washing-out the gluten. Tap water is almost invariably used yet its content of solids may vary from practically nil to 500 parts per million, and its hydrogen ion concentration may also vary widely. Most workers, however, have realised that distilled water could not be used owing to its distinct dispersive action on gluten. Wood and Hardy (1909) believed that it was the carbonic acid content of ordinary distilled water that caused the dispersion of the gluten and that this should not occur in pure water or in freshly-boiled distilled water. Le Clerc (1920), acting as referee for the Association of Official Agricultural Chemists, found that distilled water containing 0.1% of sodium chloride gave results comparable with the local tap water.

In some preliminary trials Dill and Alsberg tried the effect of using: (1) their local tap water, (2) boiled distilled water, and (3) a 0.1% salt solution. At the same time they tried the effect of varying the time taken in washing-out the gluten. Using boiled distilled water they found considerable amounts of gluten to be dispersed, the amount increasing progressively as the washing time was prolonged. The results with tap water showed that there was a progressive decrease in the non-nitrogenous, *i.e.*, the starchy, constituents of the gluten with the lengthening of the washing time but only a slight decrease in the gluten proteins, indicating that there was only slight dispersion of the gluten proteins by the local tap water. The wash water obtained after 60 minutes washing was turbid and gave a distinct test for starch. The results obtained with the 0.1% salt solution in freshly boiled distilled water were comparable with those obtained with tap water except that there was a rather more complete removal of the non-protein starchy constituents by the salt solution.

Comprehensive tests were then made by these two workers on a number of flours using as washing water: (1) tap water, (2) 0.1% sodium chloride solution, (3) 0.1% calcium chloride solution, and (4) 0.1% mixed sodium phosphate solutions of various hydrogen ion concentrations. The results showed that a 0.1% mixed sodium phosphate solution (pH about 7) resulted in the least loss of protein, this solution being slightly better than the local tap water which in its turn was slightly better than the 0.1% salt solution.

Kent-Jones and Herd (1927) examined the problem to ascertain whether the recommendations of Dill and Alsberg would ensure concordant results when glutens were washed out by various operators in different laboratories. A number of chemists accustomed to gluten washing were asked to determine the gluten contents of a number of

flours using: (1) their own local tap water, and (2) the special phosphate solution recommended by Dill and Alsberg. The work was carried out by nine different operators, each using eight different flours, the latter consisting of different grades of flour from the same grist and of straight-run flours milled from different types of wheat such as Manitoba, Australian, and Plate.

The conclusions arrived at by these workers were "that the use of a special solution, such as the one suggested by Dill and Alsberg, does not eliminate the errors inherent in gluten determinations. Even when apparently the same method is used by various operators, *i.e.*, the same amount of washing water used and the same procedure followed, there are personal differences in the manipulation of the dough and gluten which cause big variations in the results of various operators." The discrepancies referred to were large. Four workers in the same laboratory using the same flour sample, the same tap water, and the same method of work, obtained dry gluten figures differing among themselves by 2%, while the range with a single sample for all nine operators varied from 2¼ to 4% according to the type of flour. Using the standard phosphate solution only reduced the range of variation from 4 to 3%.

Berliner and Koopmann (1929) recommended using a dough made from only 10 g. of flour instead of the 20 to 33⅓ g. used by other workers. They claim a saving of time in manipulation and also greater accuracy. They realise the importance of the composition of the washing water and recommend the use of a 2% common salt solution in distilled or tap water, not because this gives more consistent results than the solutions used by the French or American chemists, but because of its simplicity in making up and because it corresponds to the amount of salt used in doughmaking in the bakehouse. A 2% solution of sodium chloride enabled gluten to be washed out from acid flours, or from those milled from unripe or frozen wheats, or from very low grade flours, from which Berliner and Koopmann could not obtain gluten by the more usual methods using tap water. These workers also recommend that instead of leaving the dough to rest under water for some time before washing out the gluten it should be worked in the hands or on a glass plate, a procedure which they state makes the resting period unnecessary.

Examining the methods used by these four sets of chemists working in France, America, England, and Germany respectively, it is found that the amount of flour used in making the dough varies from 10 g. to 33⅓ g. When the dough has been made one worker believes in washing-out the gluten at once, others believe in letting the dough remain under water for various times up to 1 hour, while others believe

in well working it with the hands. Finally, the washing water used consisted of a lime solution, a phosphate solution, tap water, or a 2% salt solution.

In spite of all this work, and much more that need not be discussed here, we still cannot answer the questions: What is the best weight of flour to use in making up the dough? What is the best treatment to give the dough prior to washing? In fact, how do each of the various factors discussed above affect the results obtained? These are some of the questions examined in the present communication.

Experimental

Kent-Jones' (1927) method of gluten washing had been used for some years in the writers' laboratories and it seemed desirable to compare other methods with it. In view of the claims made by Berliner and Koopmann their method was examined first.

In this method the dough is made from 10 g. of flour and the gluten is washed out with 2% salt solution after 1 minute's vigorous working of the dough by hand. In the writers' customary method 20 g. of flour and tap water were used and after the dough had remained for 1 hour under water the gluten was washed out with tap water. For the comparisons all glutens were washed out by the same worker so as to eliminate personal errors as far as possible.

Glutens were washed from five different flours by both methods and the results are given in Table I. In each case Berliner and Koopmann's

TABLE I

COMPARISON OF BERLINER AND KOOPMAN'S WITH KENT-JONES' METHOD OF GLUTEN WASHING

(Each figure is the average of 3 to 5 determinations.)

Flour	Method	Dry gluten (per cent of flour)	Protein in dry gluten (per cent)	Protein recovered as per cent of flour
London straight run	Berliner & Koopman	12.35	78.37	9.67
	Kent-Jones	13.13	77.61	10.20
Red Standard English	Berliner & Koopman	8.09	76.77	6.21
	Kent-Jones	8.34	79.27	6.61
No. 1 Manitoba, laboratory milled	Berliner & Koopman	12.73	78.74	10.03
	Kent-Jones	13.73	78.58	10.80
Mixed red and white English, 90% top	Berliner & Koopman	7.16	76.82	5.50
	Kent-Jones	7.60	77.96	5.92
Mixed Manitoba, 90% top	Berliner & Koopman	11.57	79.09	9.15
	Kent-Jones	12.36	80.04	9.89

method yielded less dry gluten than the other. The percentages of protein were similar in the glutens obtained by both methods showing that in the hands of the worker concerned both methods gave glutens of similar purity. The percentages of protein recovered were greater

with the Kent-Jones method showing that less gluten was dispersed or lost when this method was used.

Another worker in the writers' laboratories obtained very different results. Using Berliner and Koopmann's method he obtained glutens of protein content slightly lower than those of the first worker, but with the Kent-Jones method his glutens contained much less protein; for example, the two workers obtained from one flour glutens of 81.8 and 80.0% protein content using the former method, while using the Kent-Jones method the figures were 81.8 and 74.3% respectively. The reason for this will be explained later, but it may be concluded from these conflicting results that when different workers are washing-out glutens from different flours they are likely to obtain more concordant results and glutens of higher protein contents by Berliner and Koopmann's method than are obtained by the Kent-Jones method.

On the other hand, there appear to be objections to using 2% salt solution for gluten washing. As is well known, salt toughens the gluten and tends to make it more coherent. This explains why salt solution will enable gluten to be washed from flours which will not yield gluten at all with tap water. Such a solution tends to mask differences in gluten quality which it is the object of the test to reveal.

It seems that the solution used should be sufficiently concentrated to prevent protein dispersion with consequent loss of gluten during the washing, but sufficiently dilute to prevent any alteration or modification of gluten characters. Dill and Alsberg's 0.1% phosphate solution is an excellent standard solution with which to compare local tap waters. In most cases little difference will be found between the two, the exceptions being in those districts where the water is exceptionally soft. The St. Albans tap water is unusually hard and is entirely satisfactory for gluten washing purposes. If the local tap water is too soft, 0.1% salt solution should be used.

Treatment of the dough prior to washing-out.—The treatment given to the dough after making, but before washing-out, has a considerable effect on the amount of gluten obtained. This is illustrated in Tables II and III, which show the effect of rubbing or working the dough and of leaving it to lie under water for varying periods of time. If the gluten is washed out immediately after the dough is made, as recommended by Arpin (*loc. cit.*), less gluten is obtained than when the dough is subjected to 1 minute's working or is left to stand under water for half-an-hour. With one flour 11.73% of dry gluten was obtained when the washing was commenced immediately the dough was made; 1 minute's working increased this to 12.15%, while half-an-hour under water increased it to 12.25%. It should be stated that the rubbing, squeezing or working should be vigorous and carried out on a glass

plate in preference to between the hands as by the former means the dough can be subjected to greater pressure. Prolonging the time of working beyond 1 minute and up to 5 minutes will, with strong or moderately strong flours, increase only slightly the amount of gluten obtained. With weak flours like English less gluten is obtained after prolonged working as the dough after a few minutes' working tends

TABLE II

EFFECT OF WORKING THE DOUGH BEFORE WASHING ON THE AMOUNT AND PROTEIN CONTENT OF THE GLUTEN OBTAINED

Flour	Time of working	Dry gluten	Protein in gluten	Protein recovered
	Minutes	%	%	%
Strong	0	11.27	83.9	9.45
	1	12.25	82.3	10.08
	5	12.43	81.0	10.07
Medium	0	11.73	85.3	10.00
	1	12.15	87.4	10.62
	3	12.34	86.4	10.65
Weak	0	6.95	84.0	5.84
	1	7.31	83.6	6.09
	5	6.80	84.2	5.72

TABLE III

EFFECT OF LEAVING THE DOUGH TO STAND BEFORE WASHING ON THE AMOUNT AND PROTEIN CONTENT OF THE GLUTEN OBTAINED

Flour	Time of standing	Dry gluten	Protein in gluten	Protein recovered
	Hours	%	%	%
Strong	0.0	11.44	83.4	9.54
	0.5	12.25	84.9	10.40
	1.0	12.29	84.6	10.39
Medium	0.0	11.73	85.3	10.00
	0.5	12.25	84.9	10.40
	1.0	12.36	86.6	10.70
Weak	0.0	4.49	83.1	3.85
	0.5	6.63	85.0	5.63
	1.0	6.85	83.5	5.73

to break up in the early stages of washing, some gluten being lost in the washing water. With one strong flour the amount of dry gluten obtained was increased from 12.25% to 12.43% by increasing the time of working from 1 to 5 minutes, while similar increase in the time of treatment of a dough made from a weak flour resulted in the yield of dry gluten being lowered from 7.31% to 6.80%. Table III indicates that half-an-hour appears to be a satisfactory time to leave doughs

under water before proceeding with the washing, longer times resulting in only slightly increased yields of gluten. With one moderately strong flour 12.25% of dry gluten was obtained after half-an-hour under water, a considerable increase over the 11.73% obtained when the gluten was washed out immediately the dough was made; the yield, however, was only increased to 12.36% by leaving the dough to stand for an hour instead of half-an-hour. A comparison of Tables II, III, and IV shows that 1 minute's working and half-an-hour under water [2] yield substantially similar results. Leaving the dough to stand for half-an-hour under water tends to produce a slightly greater yield of dry gluten; but on the other hand, the glutens so obtained contain less protein than do those washed from doughs subjected to 1 minute's working. Both methods give the same recovery of protein so that the gluten obtained

TABLE IV

COMPARISON OF THE EFFECTS OF ONE MINUTE'S RUBBING AND LEAVING THE DOUGH TO STAND HALF-AN-HOUR ON THE AMOUNT AND PROTEIN CONTENT OF THE GLUTEN OBTAINED

Flour	Treatment of dough	Dry gluten	Protein in gluten	Protein recovered
		%	%	%
London straight run	None	12.10	—	—
	1 minute rubbing	12.45	84.2	10.51
	½ hour under water	12.85	82.4	10.51
No. 2 N. Manitoba	None	10.15	—	—
	1 minute rubbing	11.55	85.0	9.78
	½ hour under water	11.65	84.3	9.89
Strong short patent flour	None	10.30	—	—
	1 minute rubbing	10.65	86.1	9.21
	½ hour under water	10.95	84.0	9.17

after working is slightly purer. Working also has the advantage that no time is wasted in waiting while the dough stands under water; on the other hand, there is the disadvantage of handling with possible loss in the case of weak soft glutens due to the gluten sticking to the hand or to the glass plate. There is also a possibility of the vigorous working affecting slightly the character of the gluten although there is no evidence of this effect occurring.

The personal error in gluten washing.—Even when all the details of a method of hand washing are standardised different workers will still obtain widely varying results from the same flour. Thus two workers, A and B, in the writers' laboratories, when using the same technique obtained from the same flour 14.1% and 12.6% of dry gluten respectively. An analysis of the glutens revealed, however, that while the gluten washed out by A contained only 74.3% protein, B's gluten contained 81.8%.

[2] Leaving for half-an-hour in air covered with an inverted beaker has the same effect as standing in water for the same time. It is the resting period, not the water, that produces the effect observed.

The protein recovered in these two cases was, therefore, 10.5% in A and 10.3% in B. The actual recovery of protein was very nearly the same in both cases. In other words, both workers washed out the same amounts of protein, but A did his work less thoroughly and left more starch in his gluten. In such cases A will always obtain more dry gluten than B, but his glutens will, on the other hand, always be the poorer in protein content. This is illustrated in Table V which contains the results of gluten washings on four flours by these two workers, A and B. What is the personal factor which causes such differences in the results obtained by different workers? When a piece of dough is kneaded under a stream of water a relatively large amount of starch is washed out during the early stages and a piece of coherent gluten is obtained after 3 to 4 minutes. On analysis, however, it will be found that the gluten at this stage contains no more than about 60%

TABLE V

COMPARISON OF HAND GLUTEN WASHING BY TWO DIFFERENT WORKERS

Flour	Worker	Dry gluten	Protein in gluten	Protein recovered
		%	%	%
Red English	A	11.16	78.3	8.74
	B	10.05	83.1	8.35
No. 2 N. Manitoba	A	13.00	76.0	9.88
	B	11.86	81.6	9.68
Yeoman	A	9.67	74.5	7.20
	B	8.42	83.4	7.02
Square Head's Master	A	7.80	75.2	5.87
	B	6.92	81.9	5.67

of protein, the remaining 40% consisting of starch and other non-nitrogenous materials. If the washing is continued for a longer period the loss of these bodies continues and the percentage of protein in the gluten rises. After a time a point is reached when the loss of non-protein starchy material becomes very slow, and a slow loss of protein occurs at the same time; this is the point at which the washing is usually stopped. It is impossible to continue washing until pure, *i.e.*, 100% protein is obtained. A compromise is obviously necessary and washing is usually sufficiently complete after about 10 minutes. At this point, however, different workers will finish up with glutens of different composition, workers such as A obtaining glutens of higher starch content and therefore lower protein content and higher dry weights than workers such as B. We have arrived at the conclusion that the amount of non-protein material washed out of a dough in 10 minutes is dependent on the pressure applied to the dough during washing, a

man with strong hands squeezing more out in a given time than a man with weaker fingers. This has been demonstrated with a mechanical washer, which will be described later. The variable pressure applied to the doughs by the hands of different workers is undoubtedly the main personal factor involved in the great variations in dry gluten weights obtained by different workers from the same flour. Further, greater pressure is required and more energy is expended in working large doughs than small. In consequence, variations in this pressure factor come more into prominence with larger doughs and, as the writers have found, it is more difficult for different workers to obtain concordant results with large than with small doughs.

A Mechanical Gluten Washer

Since this personal factor is of such importance and since it is one that cannot be standardised or controlled, one is faced with the necessity of replacing hand washing by some mechanical method and it is surprising, in view of the antiquity and popularity of the gluten test, that mechanical gluten washers should have made so tardy an appearance.

A number of such machines have been developed of recent years in Europe. The one described below was developed by E. Berliner and R. Rüter and placed on the market by Messrs. Heilan, G.m.b.H., Frankfurt a/Main, under the name of the " Rotor " Gluten Washing Apparatus (Kleber-Auswasch-Apparat).

The machine consists of four curved metal blades which revolve on a vertical axis inside a cylindrical metal kneading vessel, the flat bottom of which is perforated and covered with silk gauze. The apparatus is driven by an electric motor, gearing and a revolution counter and recorder being contained in the base beneath the kneading vessel. The spindle carrying the rotating vanes also carries two small horizontal pipes connected to a small cup into which the washing water flows from a container placed above. When the machine is in action the vanes " roll and squeeze " the dough against the wall of the kneading vessel, vertical ribs on the wall holding the dough temporarily until a vane slips past, after which the next vane repeats the rolling and squeezing action. Water flows from the two tubes and plays on the dough continuously. The action is excellent and would be very difficult to improve.

The dough is made from 10 g. of flour and the washing water recommended is made from a packet of " Phossal," a mixture of salts provided with the machine. The makers' directions are that the vanes should revolve at the rate of 20 revolutions per minute, the water should

flow at 35 cc. per minute, and that washing should be continued up to 15 minutes, that is, for 300 revolutions of the vanes.

In testing this machine local tap water was used instead of a solution of " Phossal " salts, as experience had shown that this substitution would not appreciably affect the results. If the machine is to be successful it must be capable of washing-out a gluten of similar protein content to that obtained by good hand washing. Such glutens should contain 80% to 85% protein. While some hand-washed glutens have much lower protein contents than this such glutens have rather different characteristics from those richer in protein; for example, they feel stickier to the touch and when dried in the oven their colours have a distinct reddish tint due to partial dextrinisation of their starch.

Preliminary trials were carried out on a sample of straight-run flour which gave 12.1% of dry gluten by hand washing, this gluten containing 85.5% of protein. Against this, the machine as received gave 15.3% of dry gluten containing only 71% of protein. This was obtained following the makers' instructions exactly except for using tap water instead of the " Phossal " solution. Höhne (1930) states that when using this machine, together with the " Phossal " washing solution, he found 5 minutes' hand washing was necessary to remove the non-nitrogenous matter left in the gluten after 15 minutes' washing in the machine. With the flour mentioned above 1 minute's hand washing after the machine washing reduced the dry gluten percentage from 15.3 to 13.7, and 3 minutes' hand washing reduced it to 12.5, which is not very different from the figure 12.1% obtained by the customary hand washing. Five minutes' hand washing in addition to 15 minutes in the machine not only increases the time of gluten washing to 20 minutes compared with 10 minutes for normal hand washing, but the whole object of using a machine is destroyed as the personal factor is again brought into play. It was therefore decided to alter the procedure in an attempt to get glutens of high protein content by machine washing only. The makers recommend a flow of washing liquid of only 35 cc. per minute which is much slower than that usual in hand washing, but the writers found, as also did Höhne, that increasing this flow had only a small effect on the protein content of the gluten washed out. The blades of the machine as supplied are very thin and " springy " and it was evident that the pressure they put on the dough or gluten was much less than the pressure applied by the thumb during hand washing. New blades of much stouter gauge metal than those supplied with the machine were tried. These tore the dough into small bits and plastered it round the walls of the kneading chamber so that washing-out became impossible. The original blades were then used up to the point when so much of the starch is washed out that a coherent piece

of gluten is obtained, the washing being completed with the stronger blades. This procedure worked well and 150 revolutions of the weak blades, followed by 150 with the strong blades gave 14.4% dry gluten against 15.3% with 300 revolutions of the weak blades only. Increasing the number (but not the rate) of revolutions of both weak and strong blades to 200 each resulted in 13% of dry gluten being obtained, containing 80% protein (against 71% for the gluten washed out by the weak blades only). This increase of time was a disadvantage, however, so the speed of the machine was increased from 20 to 45 revolutions per minute. This not only decreased the time of washing but the extra severity of treatment given by the faster moving vanes resulted in gluten of very high protein content being washed out. Thus 200 revolutions each of both weak and strong blades resulted in 12.3% of dry gluten having the high protein content of 86%. This slight but essential modification to the machine therefore enabled it to be used as an efficient substitute for hand washing. Glutens washed out from a number of different flours had, in each case, a high protein content which was independent of the person using the machine. Replicate determinations of the dry gluten content obtained from 10 g. of flour are given in Table VI, column 2, for a number of different flours. For each flour, triplicate determinations of dry gluten content do not differ by more than 0.25%.

A Rapid Indirect Method of Determining Dry Gluten from Moist Gluten by Calculation

When washing is complete the dry weight is determined by oven drying. This drying is a long process and in an electric oven kept at 100° C. it takes 48 hours before moisture ceases to be lost. The time can be shortened by drying at a higher temperature and by pulling the gluten to pieces before putting it in the oven but in any case it is not practicable to wash out a gluten and find its dry weight in 1 day. As a consequence of this many workers use the moist weight instead of the dry weight. The moist weight is obtained by squeezing the surplus moisture out of the wet gluten with the hands which are periodically dried on a towel. This process is continued until but little more moisture can be squeezed out; the gluten is then weighed. In this state it contains about two-thirds its weight as water, the moist weight therefore being approximately three times the dry weight. Using the moist weight as an indication of the dry weight has the big disadvantage that it is very dependent on the operator. Some people squeeze much more water out of wet gluten than others. A point is reached during the "drying" when little more water can be squeezed out owing to the gluten becom-

TABLE VI

THE DRY WEIGHTS, UNDER-WATER WEIGHTS AND SPECIFIC GRAVITIES OF WET GLUTENS WASHED FROM A NUMBER OF FLOURS

Sample number	Dry weight (from 10 g. flour)	Under-water weight	Specific gravity	Average specific gravity
	Grams	*Grams*		
B. 382	1.173	0.3005	1.344	
	1.165	0.2975	1.343	1.344
	1.164	0.2995	1.346	
383	0.966	0.2410	1.330	
	0.976	0.2310	1.308	1.328
	0.980	0.2415	1.327	
384	1.115	0.2865	1.347	
	1.136	0.2915	1.345	1.346
	1.134	0.2915	1.346	
387	1.155	0.2950	1.343	
	1.171	0.2985	1.343	1.344
	1.182	0.3040	1.346	
388	1.027	0.2655	1.350	
	1.046	0.2685	1.347	1.347
	1.046	0.2665	1.344	
M. 52	1.127	0.2830	1.335	
	1.116	0.2830	1.341	1.339
	1.110	0.2815	1.340	
M. 53	1.367	0.3415	1.333	
	1.365	0.3500	1.347	1.345
	1.354	0.3555	1.355	
389	1.025	0.2685	1.356	
	1.027	0.2690	1.356	1.355
	1.030	0.2690	1.353	
390	1.077	0.2795	1.351	
	1.064	0.2775	1.353	1.350
	1.054	0.2715	1.346	

ing sticky. This sticky point is reached sooner with some people than with others, due to some condition of the hands, possibly temperature. A new method of determining the dry weight without oven drying was developed by Berliner and Rüter (1930). This method makes use of the specific gravity, S, of the dry gluten which is related to the dry weight, W_1, by the relation

$$(1) \qquad W_1 = W_2 \times \frac{S}{S-1}$$

when W_2 = weight of wet gluten under water. W_2 is quickly and easily measured. A small wire basket is suspended from the arm of a balance so that it is immersed in a beaker of water, its weight being counterpoised by the weights on the other pan of the balance. The gluten is placed in the basket and weighed. Particular care has to be taken to see that no air bubbles are attached to the gluten and the water used in the beaker should preferably have stood for some time (or better still cooled after being boiled) to allow any air bubbles to escape. The method is simple and quick but its accuracy depends on the specific gravity of wet gluten from all types of flour being the same. Berliner

and Rüter found the specific gravity of glutens washed from a few types of single wheat flours to lie between 1.30 and 1.35 with an average of 1.325. Höhne obtained an average value of 1.344. In Table VI are given the specific gravities of glutens obtained by the writers from ten different flours. These vary from 1.328 to 1.355, with an average of 1.342 which is very near Höhne's figure of 1.344. If we take 1.342 as an average figure for the specific gravity of gluten we find by using equation (1), given above, the dry weight $= 1.342/(1.342 - 1) = 3.924$ times the under-water weight. This is, therefore, the factor with which to multiply under-water weights of moist glutens to give dry gluten weights.

Höhne found the specific gravity to be influenced by the type of washing water used and by the temperature of the water under which the gluten is weighed. Since in any one laboratory the water used will not vary and the temperature of the water used in weighing can be controlled, errors due to either need only be very small. The following illustrates the much greater accuracy of the under-water weight compared with the moist weight as an indirect measure of dry weight. In a series of 32 gluten washings on four different flours the moist weight, the under-water weight, and the dry weight were measured. The ratios of dry to moist and dry to under-water weight were then calculated, the former varying from 0.306 to 0.395 with an average of 0.343, and the latter varying from 3.79 to 4.08 with an average of 3.93. The maximum variation in the ratios of dry to moist weights was 0.089 or 25.9% of the mean, while the maximum variation in the ratios of dry to under-water weights was 0.29 or only 7.4% of the mean.

From a large number of determinations it was found that for most purposes and with most flours this indirect method is reasonably accurate. However, in some circumstances some glutens behave in an anomalous manner with regard to their under-water weights. As previously stated, similar amounts of gluten may be washed from doughs that have *either* been worked vigorously for 1 minute *or* left to stand for half-an-hour under water. Such glutens should have the same specific gravity so that with either dough treatment the under-water weight should be usable as an indirect method of determining dry gluten weight. With some laboratory milled flours this has not been the case and glutens washed from these doughs which had been subjected to working or rubbing were found to have abnormally low specific gravities, the calculated dry weights in consequence being seriously inaccurate. This is shown in Table VII which contains the results of tests on four of these flours, *viz.*, three Plates and one Australian. The glutens washed from doughs which had remained under water were normal. The abnormality of the glutens from the rubbed doughs is not con-

nected with impurity of gluten as is shown by their satisfactorily high protein contents.

In view of such irregularities it is advisable when using the indirect method of estimating dry gluten weight to leave the doughs to stand under water for half-an-hour in preference to subjecting them to rubbing.

TABLE VII

EFFECT OF DOUGH TREATMENT ON THE INDIRECT DETERMINATION OF DRY GLUTEN CONTENT

Flour	Dough treatment	Dry gluten (Calculated)	Dry gluten (Found)	Protein in gluten	Protein recovered
		%	%	%	%
N.C. 110 (Plate)	Rubbing	9.1	9.6	85.7	8.19
	Standing	10.4	10.0	84.1	8.45
N.C. 116 (Plate)	Rubbing	7.1	8.6	85.8	7.37
	Standing	9.1	9.0	83.7	7.50
N.C. 117 (Plate)	Rubbing	7.6	9.9	85.3	8.43
	Standing	10.7	10.4	81.3	8.49
N.C. 119 (Australian)	Rubbing	6.0	9.2	82.6	7.58
	Standing	9.7	9.9	79.8	7.98

The flours referred to in Table VII were freshly milled at the time of the determinations. On repeating the measurements by the indirect method (with rubbing) some weeks later the samples behaved normally. Apparently the abnormality is found only in flours examined immediately after milling.

Rapid Direct Methods of Determining Gluten Dry Weight

Owing to the partial breakdown of the indirect method of determining dry gluten weight, the possibilities were examined of using higher temperatures and other methods than are usual for gluten drying. The moist glutens were cut into slices with scissors, a process which is facilitated, especially in the case of soft, sticky glutens, if they are immersed for a few minutes in boiling water before cutting up. In the water the glutens swell and lose their characteristic gluten properties; they are then easily cut up and dry very much more quickly than the unboiled glutens. For drying two ovens were available: a Hearson electric oven kept at 120° C. and a Carter-Simon oven kept at 160° C. In the Hearson oven 2½ hours is sufficient for constant weight to be attained with these cut up boiled glutens, and in the Carter-Simon oven

only half-an-hour. Drying by both these methods agrees well with drying at 100° C. for 48 hours.

Obtaining the dry weight by calculation from the under-water weight is of great value when the moist gluten is required for further work, such as swelling tests in acids, *etc.*, but on the other hand, when the dry weight only is required the new quick-drying methods give more reliable results as they are not subject to the occasional anomalies shown by the indirect method.

Method of Gluten Washing Recommended

The gluten washing machine is first set up with the weak vanes in position and the speed of the motor adjusted to rotate them at about 45 revolutions per minute. The flow of the washing water (tap water if sufficiently hard, otherwise 0.1% salt solution) should be 100 cc. per minute. The vanes, sides of kneading vessel and silk gauze in the bottom are flooded with water as, if dry, the dough will stick when operations are commenced.

Ten grams of flour are then weighed out into a small basin and by means of a long spatula doughed up with sufficient water, usually about 5.5 cc. to give a fairly slack, but not sticky, dough. This is transferred to a glass plate and rubbed or worked with the hand for 1 minute, after which it is dipped into water, to prevent sticking, and placed in the kneading vessel of the machine.

If the under-water weight is to be used as a means of arriving at the dry weight of gluten the dough should not be rubbed but left to stand under water for half-an-hour.

The dough is subjected to the action of the machine for 200 revolutions of the weak vanes, after which the machine is stopped and the vanes replaced with the stronger set (these vanes are made of Admiralty phosphor bronze of 45/1000ths of an inch in thickness), an operation which takes a few seconds only. During this change over any small particles that have been broken off from the dough are collected and united to the main bulk. The partially washed out gluten is then subjected to the action of the strong vanes for 200 revolutions, after which washing is complete and any small particles that have been broken off are collected and united with the main piece of gluten.

To determine the dry weight by the indirect method of weighing under water, a small container made from a piece of wire gauze or perforated metal sheet is suspended by fine wire from one arm of a balance underneath water in a beaker placed on a wood bridge spanning the pan of the balance. The water in the beaker should contain no air bubbles, preferably being cold, recently boiled water. The under-

water weight of the container is determined (or, the container should be counterpoised), after which the gluten should be squeezed under water to dislodge any adhering air bubbles and then transferred to the container and weighed. The under-water weight of the gluten multiplied by the factor 3.92 gives the dry weight of gluten washed from 10 g. of flour.

To determine the gluten dry weight by direct weighing of the oven-dried gluten, the gluten is first dropped into boiling water for 2 or 3 minutes and after cooling in cold water is cut up into small pieces with scissors into a previously weighed tin. This is then placed in the oven and dried to constant weight, the time taken depending on the oven used. With a Hearson electric oven, kept at 120° C., the time required is about 2½ hours, and with a Carter-Simon oven kept at 160° C. about half-an-hour. After drying, the containers, plus gluten, are cooled in a desiccator for 15 minutes and weighed.

Conclusions

Some earlier work on the gluten washing problem is reviewed. The principal factors affecting the accuracy of gluten determinations made by hand working are: (1) the amount of flour used; (2) the composition of the water used in washing; (3) the temperature of the water; (4) the treatment of the dough prior to washing out; i.e., (a) the extraction is done immediately after dough-making, or (b) the dough is allowed to rest under water, or (c) the dough is worked vigorously for a short time without any resting period; (5) the duration of the washing; (6) a personal factor characteristic of each operator.

The personal factor, which is by far the most important of the above, can be eliminated by using a mechanical means of gluten washing. One such instrument developed for this purpose and called the "Rotor" Gluten Washing Apparatus is described.

A rapid method of determining dry gluten from wet gluten by calculation from the under water weight developed by Berliner and Rüter is described.

Rapid methods of gluten drying at relatively high temperatures have been devised that enable dry gluten figures to be obtained in one hour by direct oven drying.

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SOLUBLE AMYLO-PHOSPHORIC ACIDS AND THE NATURE OF THE PASTE-FORMING STARCH FRACTIONS

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Many investigators have verified our theory concerning the occurrence of organically linked phosphoric acid in starch. Some authors, however, have not noted, or at least have not correctly valued some observations. Since some discussions have arisen, the purpose of this paper will be to explain some essential facts, and add details which have not been published heretofore.

The relative quantity of phosphoric acid in potato starch is very small. First, it is necessary to clarify the question as to whether the small quantity of phosphoric acid present is combined with the whole of the starch substance or with only a part of it. To do this one must attempt to separate potato starch into two fractions, one containing phosphorus and one having no phosphorus. For this purpose, to date, two methods have usually been employed, which are as follows:

(1) The starch granules are cautiously swollen. A small quantity of the starch substance becomes soluble, but the greater part of the starch is in the form of greatly swollen granule fragments. Nearly the whole of the phosphoric acid is found in this gel-like residue.

(2) The starch is treated with water at 120° C. in an auto-clave for a short time, and the solution obtained is then electrodialyzed. The gel fraction which separates contains practically all of the phosphoric acid, while the sol substance is nearly free of phosphorus.

If, however, the swelling process is not performed cautiously, the sol phase will also contain some phosphoric acid, and the same is true if the starch solution is heated for too long a time under pressure at 120° C. before being subjected to electrodialysis.

It follows, then, that the presence of phosphoric acid does not necessarily render the starch substance insoluble, since the conditions of the experiment determine whether the amylo-phosphoric acid is obtained in the form of a gel or a sol. Some examples of the results of our researches on this point are given in Table I.

Similar observations by Malfitano (1906), Sherman and Baker

TABLE I

PHOSPHORIC ACID CONTENT OF HIGHLY PURIFIED GELS AND SOLS PREPARED FROM POTATO STARCH

Method of separation	P_2O_5 content In gel %	P_2O_5 content In sol %
Swollen in 1% alkali, precipitated with acetic acid	0.167	0.134
Pasted with water (Samec, 1914)	0.147	0.045
Swollen with 0.6% alkali, precipitated with sulfuric acid (Samec, 1914)	0.185	0.007
Auto-claved ½ hour at 120° C., electrodialyzed	0.175	0.004
Auto-claved 6 hours at 120° C., electrodialyzed (Samec and Mayer, 1922)	.009	0.015
	Top layer	Bottom
Soluble starch, Lintner (Samec and Jencic, 1915)		0.111
Filtered through collodium membrane (Wolff-Fernbach starch) (Samec, 1931)		0.120

(1916), Karrer and Krauss (1929), Baldwin (1930), Hirst, Plant, and Wilkinson (1932), and Schoch (1933) could be added to the above table.

By prolonged treatment of the starch granules with hot water, or by repeated boiling and electrodialyzation, it is possible to obtain the whole of the starch substance, including the phosphoric acid, in form of a sol. This sol, by further treatment, can be separated into a series of starch fractions. Until now, we have not known what mutual relationship existed between the polysaccharid and the phosphoric acid when in the sol form, nor have we known the distribution of the phosphoric acid in the fractions giving different colors with iodine (amylo and erythro-amyloses). The following experiments give an answer to this question.

It was found necessary in these experiments to remove the inorganic phosphates, which may be formed during the solving process, in order that our conclusions would be free from this objection. This possibility has been overlooked by some investigators, and many deviations in their results can be explained by the error due to inorganic phosphates. We have given this problem very careful attention and the sols used for this investigation were highly purified.

Experimental

I. Solving by Heating Under Pressure

We heated a portion of an approximately 2% potato starch paste ½, 3, and 5 hours at 120° C. The concentration and the quantity of P_2O_5 in the whole solution were then determined. The solution was then electrodialyzed, and the concentration and P_2O_5 content of each of the two phases were determined. Finally, we tested the fractions with iodine, using a colorimeter, and noted the effect of adding increasing increments of iodine. We obtained thus the data given in Table II.

The first marked chemical change that the starch undergoes, when

heated under pressure, consists in the separation or loss of phosphoric acid. After 5 hours of heating the starch contained only 30% of its original phosphoric acid content. Although the greater part of the P_2O_5 separates with the gel phase (about 95%), nevertheless, about 5% of the total phosphoric acid is combined in organic linkage in the sol phase.

At the same time the relative quantity of the sol phase (or the substance not separated by electrodialysis) increases from 19.5 to 25.7 (*e.g.*, increases 6.2%) as the time of heating is increased from ½ hour to 5 hours. From these figures it follows, that 100 parts of the newly

TABLE II

PHOSPHORUS CONTENT AND IODINE COLOR OF GELS AND SOLS, OBTAINED FROM POTATO STARCH PASTES, HEATED AT 120° C. FOR DIFFERENT PERIODS OF TIME

Time of heating in hours	½	3	5
	Grams	*Grams*	*Grams*
P_2O_5 in 100 g. dried starch substance (Before electrodialysis)	0.142	0.090	0.043
P_2O_5 in 100 g. of sol substance	0.004	0.023	0.008
P_2O_5 in 100 g. of gel substance	0.175	0.108	0.055
From 100 g. of starch, one obtains			
as sol	19.5	20.8	25.7
as gel	80.5	79.2	74.3
From 100 g. P_2O_5, one obtains			
in the sol	0.55	5.3	4.8
in the gel	99.45	94.7	95.2
Iodine color of sol[1]	Blue-green	Blue-green	Blue-green
Iodine color of the gel	Blue-violet red	Blue-violet orange	Blue-violet orange

formed starch substance not precipitated by electrodialysis corresponds to 0.032 part of P_2O_5, which is only a little smaller value than that corresponding to the P_2O_5 content of the gel (0.055%).

The iodine color of the sols was always clear blue and changed into green with an excess of iodine.

From these data, it follows that with the heating of a solution of potato starch under pressure, phosphoric acid is separated from the starch micelles. The residue of phosphoric acid is still great enough after 5 hours heating to cause precipitation by electrodialysis of the greater part of the starch. Of the paste-forming material (gel), a relatively small quantity of a phosphoric-polysaccharid separates further, the organic component of which belongs to the group of amylo substances.

In the gel-phase, obtained after ½ hour heating at 120° C., 1 gram atom of phosphorus corresponds to about 40,500 grams of organic

[1] The first color occurs after addition of a small quantity of iodine, the second on saturation with iodine. Dubosque colorimeter. Starch solution 0.03%; 1/100 N Iodine in KI solution.

substance. After 5 hours of heating this relation changes to 1 gram atom of phosphorus to 142,000 grams organic substance. If we estimate the average size molate of starch in the gel phase, then M = 207,000 to 230,000. In the gel obtained after heating ½ hour we have one molecular aggregate combined with five phosphoric acid residues, while in the gel obtained after 5 hours heating we have one molecular aggregate combined with 1.5–2.0 phosphoric acid residues.

The phosphorus-containing component of the sol phase would contain, by this same method of estimation, one phosphoric acid to about 237,000 parts of organic substance. In the case of the sol, evidently, the electrical charge is not sufficient to permit separation by electrodialysis.

II. Fractionation of the Starch Obtained by the Method of Wolff-Fernbach

A longer period of heating under pressure separates further quantities of inorganic phosphoric acid, and therefore the preceding method is without value for isolation of soluble amylo-phosphoric acids.

We, therefore, prepared a soluble starch after the method of Wolff and Fernbach (1905) and used it to study the distribution of phosphorus in different fractions. When this starch is heated ½ hour with water at 120° C. one obtains a solution which contains all the phosphorus of the starch. With electrodialysis, the inorganic phosphoric acid and the carbohydrate-phosphoric acid compounds, which pass through parchment, migrate to the anodic tube. We have in this way isolated in the anodic tube an organic phosphoric acid with 2.9% $P_2O_5[(C_6H_{10}O_5)_{15} \times H_3PO_4]$ (Samec, 1931). During electrodialysis there is no separation into fractions so that the non-dialyzable residue consists of fractions with and without phosphorus.

For separation, we tried different methods of fractionation. For our problem we obtained the best success with the partial separation with 40% alcohol. After the addition of alcohol a part of the substance soon separates as a flocculent precipitate (I); another part remains in the form of a suspension and can be removed by centrifugation (II). If the solution is evaporated to one-half the original volume a filterable precipitate separates (III); a further part can be removed only by centrifugation (IV); and finally there still remains in the solution a considerable quantity of the original (V). Table III shows the distribution of the total starch substance in these five fractions, and their P_2O_5 content.

The separation of fractions without phosphorus from fractions with phosphorus was not successful by this method, but one does obtain two principal fractions (IV and V) containing very different quantities of phosphorus. In general, the fractions, which are precipitated by alcohol, contain only a small quantity of phosphoric acid, while the

fraction remaining in solution and comprising 40% of the whole, contained 0.175% P_2O_5. The same relative quantity of P_2O_5 is found in the gel, which separates when a potato starch solution heated ½ hour at 120° C. is subjected to electrodialysis. (See Table II.) Schoch also found the same relative quantity of phosphorus $\left(\begin{matrix}0.175\%\ P_2O_5\\ .076\%\ P\end{matrix}\right)$ in the anodic tube fraction obtained by an electrophoretical fractionation of native and ground potato starch.

TABLE III

PHOSPHORUS CONTENT OF DIFFERENT FRACTIONS OBTAINED BY PRECIPITATION WITH ALCOHOL (40%) OF A SOLUTION OF STARCH PREPARED AFTER THE METHOD OF WOLFF-FERNBACH

	Percent of whole starch substance	P_2O_5 in 100 g. of the dry substance
		Grams
Original starch in solution	100	0.113
Precipitate with 40% alcohol (I)	0.57	0.027
Substance obtained by centrifuging of the mixture with 40% alcohol (II)	14.3	0.060
Coagulum after partial evaporation (III)	5.65	0.068
Substances obtained by centrifuging after partial evaporation (IV)	49.77	0.047
Remaining in solution	41.29	0.175
Lost	1.29	

III. Fractionation of Ground Starch

The capacity of a starch to form paste decreases with continued grinding of the starch and the grinding also peptizes the starch to such an extent that it can pass through ultra-filters (Zwikker (1921), Schoch (1933)). The fraction which passes through the ultra-filter does not differ in phosphorus content from the original substance, but it is possible to separate solutions of ground starch by electrodialysis into different fractions.

We disintegrated potato starch by grinding for 500 hours in a china pebble mill. A 2% paste was prepared at 85° C. and heated ½ hour at 120° C. This was then filtered through filter paper and electrodialyzed until the conductivity of the liquid in the anodic tube became constant. A double layer without sharply defined limits appeared. The upper liquid was weakly yellow and clear, the middle layer was light brown and clear, and the solution beneath was dirty brown and cloudy. The distribution of phosphorus in these phases is given in Table IV.

Here, also, we find fractions with different phosphorus content and a large part of the phosphorus is found in the sol substance.

TABLE IV

FRACTIONATION BY ELECTRODIALYSIS AND PHOSPHORUS CONTENT OF SOLUTIONS OBTAINED FROM POTATO STARCH GROUND 500 HOURS

	Upper layer	Middle layer	Lower layer
Concentration, %	1.18	4.57	6.92
P_2O_5 in 100 g. dried substance	0.032	0.203	0.055

Discussion

The classification of the constituents of starch granules follows in general the two outlines given below:

(1) Soluble parts (amyloses, *B*-amyloses); insoluble (or very difficultly soluble amylocellulose).[2]

(2) Soluble parts not forming paste (amyloses); paste forming parts (amylo-pectin).[2]

We have stated previously the theory that the paste forming starch component consists of a carbohydrate in combination with phosphoric acid (Samec, 1912). Some authors believed that the discovery of soluble carbohydrate-phosphoric acids was an argument against this theory. We can show by several examples that this is an incorrect interpretation, and wish to emphasize that the presence of an organic phosphoric acid is an essential, but not the only factor in causing the starch to form paste. (Samec and Mayer, 1922; Samec, 1930, 1931, 1931a, 1931b, 1933, 1933a).

The circumstances can be represented more exactly as follows: The principal mass of most species of starch is in the form of a polysaccharid, water solutions of which give with iodine a clear blue solution and a green color with an excess of iodine: amylose, amylo-amyloses (Samec and Mayer, 1921). There is also present in the starch granule in smaller quantities a polysaccharid, which in dilute water solution becomes violet to violet red with a small amount of iodine, and red with an excess of iodine (Samec and Mayer, 1921).

In starch from some species of plants these two starch components are combined with phosphoric acid (Samec, 1912; Samec and Haerdtl, 1919; Samec, 1927; Samec and Klemen, 1931; in starches from other species of plants these two starch components are linked with fatty acids (Taylor and Nelson, 1920; Taylor and Lehrman, 1926; Taylor and Werntz, 1927; Lehrman, 1929, 1930, and 1932; Lehrman and Kabat, 1932; Taylor and Sherman, 1933; Samec, 1931; Taylor and Walton, 1929). In some cases the starch is combined with a substance containing both nitrogen and phosphorus (Samec, 1927, 1928; Samec and Blinc, 1929; Samec, 1931). Silicic acid is also always present in the starch

[2] Some authors use the terms amylo-cellulose and amylo-pectin synonymously. In my opinion this is not to be recommended.

granule (Samec, 1914; Malfitano and Catoire, 1922; Ling and Nanji, 1925; Karrer and Krauss, 1929; Strohecker, Vaubel, and Breitweiser, 1935).

The colloidal behavior of starch is influenced by the forces exerted (1) by association of molecules, and (2) by solvation of molecules (hydration).

These two forces are expressed rather differently in the two starch components which form the starch granule. The amylo-amyloses possess a great tendency towards association, and they also show a great tendency to hydrate. In water solution, the concentration is low (0.3–0.59) and they are stable for only a few days, since the sols soon become cloudy and most of the sol separates as a white much swollen coagulum. When dried the substance is horny and difficultly soluble in water.

In the erythro-amyloses the forces of molecular attraction are not great, and this substance is not so highly hydrated. The sols are stable and also have a higher concentration than those of the amylo-amyloses and the coagula is very soluble (Samec and Mayer, 1921a; Samec and Blinc, 1929; Samec, 1931).

Esterification with phosphoric acid, thus introducing a group with ability to ionize, greatly increases the hydration capacity and does not essentially decrease the tendency of molecules to associate (Samec and Mayer, 1921a, 1922; Samec, 1924).

Careful researches concerning the colloidal changes which occur due to esterification of the starch substance with fatty acids are not as yet available, but we assume that the hydration capacity will thereby be decreased since longer chains of $C-H_2$ are introduced into the molecule. The hydration capacity is also decreased by compounds containing phosphorus and nitrogen.

For the formation of a paste, it is necessary to have a proper balance between the association and hydration forces. On account of their great tendency to associate, the amylo-amyloses do not form pastes. Dilute sols form a thin liquid and as concentrations are increased they ecome flocculent. When esterified with phosphoric acid, their hydration capacity increases to such an extent that at a concentration of 0.5% (dry substance) they form with water clear, sticky jellies. (Samec and Mayer, 1922). To obtain a real paste the association forces of the amylose must be decreased to the proper point. With potato starch and starch from related species such as curcuma, canna, *etc.*, this is accomplished by the presence of erythro-amylose with the amylo-amyloses. The mixture of these two starch substances, when esterified with a small quantity of phosphoric acid, possess the pasty, slimy consistency (mucilaginous) which caused Maquenne to use the pression *amylopectin*.

We can, however, decrease the tendency towards association by addition of other substances (*e.g.*, by small quantities of alcohol) and we may increase hydration by the addition of alkalies instead of esterification with phosphoric acid. In this way amyloses acquire the capacity to form pastes and can be drawn into long threads. Some salts, especially, are capable of influencing the powers of molecular attraction and also draw water to the surface of the micelles. Rhodanids (thiocynates) and borates, in particular, make possible the drawing of threads several meters in length from dilute amylose pastes.

In wheat starch the paste-forming components consist of amylo-amyloses, erythro-amyloses, and a compound containing phosphorus and nitrogen. The effect of phosphoric acid is greatly decreased by the nitrogenous component, and the influence of phosphoric acid in increasing hydration is not as marked as in potato and similar starches. Pastes from wheat starches are white, cloudy, and a little slimy. The starches of cereals do not contain as large quantities of phosphorus, but contain fatty acids. Pastes from starch of such starch are "pasty" and not "pectinous."

On account of the strong molecular attraction the amyloses in water are not in the form of simple molecules, but in the form of molecular aggregates (molates or micelles). A gel results at small concentrations since the starch is present as molecular aggregates. Therefore, it is clear that with decreasing size of molates or micelles, the tendency to form paste decreases. We observe a "liquefying" of pastes by continued heating of the starch parts at higher temperatures. With certain starch preparations, *i.e.*, washing the granules with dilute acids (Lintner, 1886; Wolff-Fernbach, 1905; and Schoch, 1933); exposure to ultra-violet rays, (Samec, 1926); severe grinding and other methods, the granule constituents have been so altered that they cannot form pastes. It is clear, therefore, that the ability to form paste decreases if the glucosidic linkage is disturbed.

Both of these two latter methods of peptization mentioned above may be accomplished without separation of the phosphoric acid.

We often find phosphoric acid linked with smaller organic molecules. The existence of such compounds is a confirmation rather than a negation of our theory, that phosphoric acid is organically linked in the starch molecule.

Conclusion

Potato starch and related species of starch contain organically linked phosphoric acid. When starch is pasted, the phosphoric acid is present in the paste forming fractions of the starch.

It is also possible to obtain water soluble amylo-phosphoric acids by washing the starch granules with dilute acids, by prolonged heating of

starch pastes, by severe grinding of the starch granules, by exposure to ultra-violet rays, and by suitable hydrolysis of starch with diastase. In the starch solutions prepared in such manner, amylophosphoric acids are present, having micelles of different size and different contents of phosphoric acid.

Paste formation depends on having a proper balance between the tendency to associate and to hydrate. With potato starch, and starches from similar species, this relationship is attained by the presence of both amylo-amylose and erythro-amylose, and by esterification of the polysaccharid with phosphoric acid.

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REPORT OF THE 1934–35 COMMITTEE ON CAKE-BAKING TESTS

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(Read at the Annual Meeting, June 1935)

The reports presented by members of the Committee on Cake-Baking Tests lead to several recommendations for the attention of the subsequent Committee.

The report read by W. E. Stokes indicates that Supplement A is best conducted by adding the sugar increments in dry form instead of in solution.

The Committee recommends the use of photographs, similar to those shown and described by J. R. Davies, in conjunction with the Standard cake score, to record and preserve records of cake-baking tests. It is also suggested that a study be made, leading to the preparation of type photographs, to be used as an aid in classifying external and internal characteristics of test cakes. This study would be parallel to the study conducted some years ago for a similar purpose in the case of "pup" loaves.

H. R. Fisher has shown, by his study of the mixing procedure, that the results obtained through the use of varying sizes of bowls, and by beaters and whips, will vary. Therefore, beaters and whips are not interchangeable, and the bowl size and beater specified in the Standard Cake Baking Method must be adhered to for standard results.

The work by Mr. Fisher was conducted at a high altitude, in Idaho, and indicates the need for preparing a chart which will show the necessary adjustment of the quantity of soda and cream of tartar for different atmospheric pressures. This chart would be for the use of laboratories working in the higher altitudes.

The specifications suggested by C. A. Glabau should be adhered to when securing dried egg albumin for use in the Standard Cake Baking Test. The Committee recommends that intensive collaborative work should be conducted next year in order to finish as soon as possible the study of albumin. This appears to be the most important single problem now faced by the Committee. Also, in view of the fact that many technicians have had difficulty in making satisfactory test cakes with dried albumin, the Committee suggests the alternative use of fresh or frozen egg albumin in the standard formula, provided that the type of albumin be described in connection with the score.

THE RELATIVE MERITS OF THE BEATER AND WHIP AND OF SIZE OF MIXING BOWL ON THE RESULTS OBTAINED WITH THE A. A. C. C. BASIC CAKE-BAKING METHOD [1]

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(Read at the Annual Meeting, June 1935)

The problem assigned was to determine the relative merits of the beater and whip, and of size of mixing bowl upon the results obtained with the A. A. C. C. basic cake-baking method.

Two sizes of bowls were used—a Monel metal 10-quart bowl equipped with a Monel metal wire whip and a bronze beater, and the standard 3-quart bowl, of nickel, also equipped with a Monel metal wire whip and a bronze beater.

The mixer used was a Hobart model E-10 with standard equipment for the 10-quart bowl. An adapter was used with this machine for the 3-quart bowl. The mixer had an arm speed of low, 64 r.p.m.; medium, 112 r.p.m.; and high, 200 r.p.m.

Two flours were used which were known to vary considerably under commercial practices, in their cake-making ability. One flour was a commercially milled cake flour. The other flour was a fancy soft clear. Both flours were milled from soft white wheat. The cake flour was bleached with Beta Chlora, and the soft clear was bleached by the Alsop process. A chemical analysis of the flours is given in Table I.

TABLE I

CHEMICAL ANALYSIS OF FLOURS

	Moisture	Ash	Protein	pH
	%	%	%	
Soft clear	15.0	.528	8.50	5.80
Cake flour	15.0	.360	7.05	5.06

The oven used was a Westinghouse commercial-type 10-loaf oven.

The basic cake-baking formula was used with both flours, with one exception—the amounts of soda and cream of tartar were reduced.

[1] Subcommittee report of the 1934–35 Committee on Cake Baking Test.

In the preliminary bakings it was apparent that the leavening was too great for this altitude (3780 ft.). Cakes baked with the full amount of soda and cream of tartar were very coarse, having a harsh, dry texture. Through several trials it was found that a reduction of 16.6% in both the soda and cream of tartar gave considerably better cakes. This reduction was also applied to batters on which the specific gravity only was determined.

The study originally contemplated the use of four types of flours: a cake flour, an unbleached cake flour, a straight grade soft patent flour, and a fancy soft clear flour. It was believed that such a group of flours would cover practically all of the types of flours used for cake baking. Due to the limited time available for the work it was decided to eliminate the unbleached cake flour and the soft patent flour from the study and to just use the two extremes in flour quality.

For some reason, as yet undetermined, only the clear flour made a smooth batter. Changing the formula by adding the soda last did not seem to help. Reducing the liquid helped to a certain degree, but this did not entirely overcome the slightly curdled batters. Slight variations in the temperature of the shortening also proved unsuccessful.

There was available only one batch of egg albumin with which all the tests were made. It is very possible that a part of the curdling difficulty might have been due to egg albumin which was not suitable, as fresh eggs improved the appearance of the batter.

In the baking experiments the basic procedure was followed, with the one exception noted. However, the mixing time, at medium speed, was varied to include specific gravity tests and bakings after 1, 5, and 10-minute mixing periods. The specific gravities were taken just prior to adding the cream of tartar. The specific gravities of the clear flour were made with a small picnometer.

The results obtained with the clear flour are tabulated in Table II.

In mixing it was noted that the whip gave a smoother appearing batter in each case than the corresponding beater. In this series the combination of 10-quart bowl and wire whip gave the smoothest appearing batter, although the 3-quart bowl and wire whip gave the lightest batter.

The cakes made with the 3-quart bowl and wire whip fell when taken from the oven, with the exception of the cake mixed one minute. This was probably due to having a batter too light considering the flour and amount of leavening agents used. The specific gravities would indicate that several other combinations should have fallen as well. This could be accounted for in the possibility of a somewhat better bake on these other combinations. The whip and 3-quart bowl batters, mixed for 5 minutes, fell but very little. The whip and 3-quart bowl batters mixed

TABLE II

RESULTS OF BAKING TESTS AND SPECIFIC GRAVITY READINGS ON BATTERS USING THE CLEAR FLOUR LISTED IN TABLE I

	Ideal score	Mixing device											
		Small beater [1]			Small whip [1]			Large beater [2]			Large whip [2]		
		Mixing time			Mixing time			Mixing time			Mixing time		
		1 min.	5 min.	10 min.	1 min.	5 min.	10 min.	1 min.	5 min.	10 min.	1 min.	5 min.	10 min.
		Score	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*
External appearance													
Symmetry	10	10	9	9	10	5	3	9	10	7	9	7	10
Volume	15	10	10.5	10.8	9.9	10	10	10.5	11.9	11.2	10	11.1	11.2
Crust	5	3	3	3	3	3	3	3	3	3	3	3	3
Internal appearance													
Texture	30	15	17	17	13.5	15	9	15	19	19	15	19	19
Grain	25	17.5	19	19	15	17.5	11	17.5	21	21	20	22.5	22.5
Color	15	6	6	6	6	6	6	6	6	6	6	6	6
Total score	100	61.5	64.5	64.8	57.4	56.5	42	60.5	70.9	67.2	63	68.5	71.2
		Specific gravity of batters											
Before adding cream of tartar		1.173	1.079	1.039	1.114	1.033	.988	1.121	1.057	1.033	1.077	1.047	1.002

[1] 3-quart bowl.
[2] 10-quart bowl.

for 10 minutes definitely fell and it is believed that no baking differences could have altered this.

In pouring the batters from the mixing bowls it was noted that the 3-quart bowl and beater gave a batter of such consistency that two cakes were easily poured without scraping the bowl. In the case of the 3-quart bowl and whip batters it was just possible to pour two cakes. With the larger bowl batters using the beater, it was possible to pour only one cake and about a third of the batter for the second cake. With the 10-quart bowl and whip batters one cake was about all that could be poured. This can be accounted for by the fact that the beater and whip with the 10-quart bowl, travel at a higher speed due to their greater size and as a result the batter was kept away from the bottom of the bowl and a great deal more of the side surfaces were covered with batter. The greater surface covered would not permit the batter to flow from the bowl.

The soft clear flour from which these bakings were made was best suited for the type of goods in which spread is important. Under commercial conditions it is not considered a good flour for cakes. This flour under commercial conditions almost invariably gives cakes dark in color, soggy, and poor in every respect.

In Table III are tabulated the results obtained with the cake flour. This flour was opposite in baking characteristics to the soft clear flour—making excellent cakes and poor sugar cookies, or similar goods.

The pouring characteristics of batters made from the cake flour were similar to the clear flour. It was not possible to pour two cakes from batters made in the 10-quart bowl, for reasons outlined above.

In appearance, the batters made with the 10-quart bowl and whip seemed the smoothest, approaching the clear flour batters in that respect. None of the cake-flour batters, however, was entirely smooth. As Table III indicates, with the exception of the batter mixed 1 minute, the cakes were almost identical. None of the cakes in this series fell. The cakes were not on a par with cakes baked using various commercial formulae.

The specific gravity determinations in this series were made by weighing small vaccine bottles filled with batter. This method was considered more accurate and faster than using a picnometer, which tends to give high specific gravities as it is necessary to force the stopper in the bottle, thus compressing the batter.

Specific gravities were taken before and after adding the cream of tartar. Those specific gravities taken after the cream of tartar was added are not very significant as the time factor was so important it was impossible to get comparative results.

TABLE III

RESULTS OF BAKING TESTS AND SPECIFIC GRAVITY READINGS ON BATTERS USING THE CAKE FLOUR LISTED IN TABLE I

	Ideal score	Mixing device											
		Small beater [1]			Small whip [1]			Large beater [2]			Large whip [2]		
		Mixing time			Mixing time			Mixing time			Mixing time		
		1 min.	5 min.	10 min.	1 min.	5 min.	10 min.	1 min.	5 min.	10 min.	1 min.	5 min.	10 min.
		Score	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*
External appearance													
Symmetry	10	10	10	10	10	10	10	10	10	10	10	10	9
Volume	15	10	11	11.3	9.8	11.3	11.2	10.1	11.8	11.1	9.5	11.7	11.6
Crust	5	4	4	4	4	4	4	4	4	4	4	4	4
Internal appearance													
Texture	30	20	26	26	20	26	26	22	26	26	22	26	26
Grain	25	12	20	20	12	20	20	16	20	20	18	20	20
Color	15	13	15	15	13	15	15	13	15	15	13	15	15
Total score	100	69	86	86.3	68.8	86.3	86.2	75.1	86.8	86.1	76.5	86.7	85.6
		Specific gravity of batters											
Before adding cream of tartar		1.160	.871	.900	1.095	.870	.880	1.103	.912	.914	1.043	.893	.894
After adding cream of tartar		1.066	.859	.840	.988	.838	.841	.996	.871	.877	.962	.859	.855

[1] 3-quart bowl.
[2] 10-quart bowl.

In view of the results obtained from the baking tests it was decided to make an additional series of specific gravity determinations, which promised to show differences of a greater degree than was noted in the cakes. The results obtained are tabulated in Table IV. The soda was also reduced 16.6% in these batters. In the case of the cake flour the water was increased 10% to give a batter of approximately the same consistency as the clear flour.

TABLE IV

SPECIFIC GRAVITY OF CAKE BATTERS[1]

Mixing time	2M	4M	6M	8M	10M	12M	16M	20M
				Fancy Soft Clear Flour				
Small beater	1.033	0.977	0.962	0.959	0.944	0.945	0.920	0.910
Small whip	.961	.931	.925	.908	.896	.889	.877	.874
Large beater	.988	.969	.954	.940	.946	.934	.931	.932
Large whip	.974	.966	.954	.939	.927	.924	.922	.926
				Cake Flour				
Small beater	1.085	0.986	0.949	0.924	0.912	0.912	0.903	0.908
Small whip	.983	.898	.879	.866	.865	.867	.869	.865
Large beater	.999	.929	.911	.911	.906	.902	.909	.913
Large whip	.927	.898	.902	.902	.898	.892	.892	.880

[1] These determinations were made by filling a small, approximately 20 cc., vaccine bottle and weighing. The A. A. C. C. basic formula was used except with the cake flour which was made with 10% extra liquid and 2.5 g. of soda instead of 3 g.

It will be noticed that in the early stages of mixing there were quite large differences in the specific gravities of the batters. These differences grew less as the mixing time increased. The groupings are fairly close as the mixing time nears 10 minutes, with the exception of the 3-quart bowl and wire whip mixes, which gave consistently lighter batters throughout. The 3-quart bowl combination seems to be the least efficient.

The work would indicate that beaters and whips are not interchangeable, particularly in the 3-quart bowl.

The 10-quart bowl is not satisfactory when using the basic formula as it is not possible to pour two batters without scraping. This bowl is also unsatisfactory because the larger equipment introduces a higher speed to the mixing operation.

The wire whip appears to be a more efficient means of mixing than the beater.

THE SUGAR AND SHORTENING TOLERANCE OF SOFT WHEAT FLOURS FOR CAKE MAKING[1]

LYDIA COOLEY and J. R. DAVIES

Calumet Baking Powder Company, Chicago, Illinois

(Read at the Annual Meeting, June 1935)

Our limited investigations using the formula and following the procedure outlined in the A. A. C. C. basic cake-baking test have shown us the necessity of supplementary tests A (sugar tolerance) and B (shortening tolerance) in order to evaluate the cake-baking qualities of flour.

Bearing on this point are some data from a number of experiments made in our laboratories.

Two commercially milled flours—a short patent and a fancy clear, and an experimentally milled flour were used in our studies. The chemical analyses of the commercially milled flours are given as Table I.

TABLE I

CHEMICAL ANALYSES OF THE COMMERCIALLY MILLED FLOURS (15% MOISTURE BASIS)

	Ash	Protein	pH	Viscosity
	%	%		°MacM.
Fancy clear	0.400	9.40	5.30	44
Short patent	0.325	9.25	5.20	52

In making tests we departed somewhat from the A. A. C. C. procedure in that we used smaller cake pans with a corresponding decrease in weight of batter. The pans were $3\frac{1}{2} \times 6 \times 2\frac{1}{4}$ inches in size. 265 g. of batter was used. The baking was done in a rotary electric oven at 375° F. for 30 minutes.

The results of the baking tests, in terms of loaf volume and specific volume (loaf volume divided by weight of cake), are given in Table II.

From an examination of the data shown in Table II it is apparent that supplementary test A is a more drastic test of the cake-baking quality of a flour than is test B. The results of these tests are best shown, however, by the photographs of the cakes exhibited as Figures 1, 2, 3, 4, and 5.

[1] Subcommittee report of the 1934–35 Committee on Cake Baking Test.

TABLE II

EFFECT OF INCREASING QUANTITIES OF SHORTENING AND SUGAR ON THE LOAF VOLUME AND SPECIFIC VOLUME OF CAKE MADE FROM DIFFERENT GRADES OF FLOUR

Treatment	Fancy patent flour		Short patent flour	
	Loaf volume	Specific volume	Loaf volume	Specific volume
	Cc.		*Cc.*	
Supplement A				
Control [1]	579	2.52	574	2.49
10% increase in sugar	550	2.38	561	2.41
20% " " "	520	2.27	539	2.32
30% " " "	487	2.22	520	2.23
Supplement B				
Control [1]	594	2.55	572	2.47
25% increase in shortening	560	2.42	547	2.36
50% " " "	529	2.29	516	2.21

[1] A. A. C. C. basic cake formula.

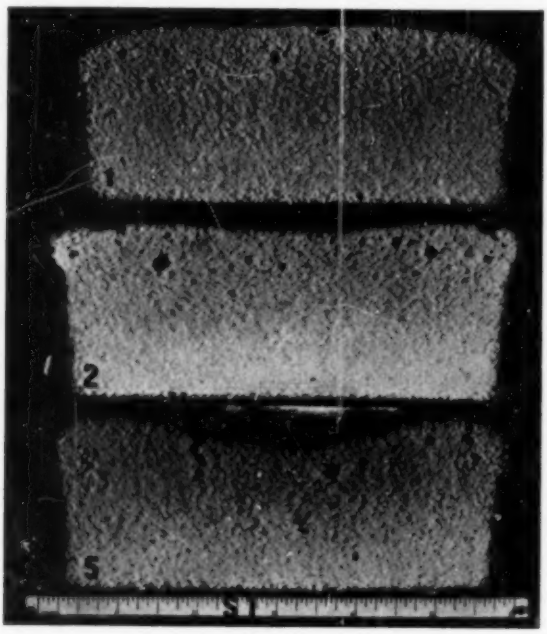

Fig. 1. Experimentally milled flour.
Cake No. 1—Control
Cake No. 2—Shortening tolerance
Cake No. 5—Sugar tolerance

The experimentally milled flour (Figure 1, cake No. 5) showed a sugar tolerance of less than 10%, the fancy clear (Figure 5, cake No. 6)

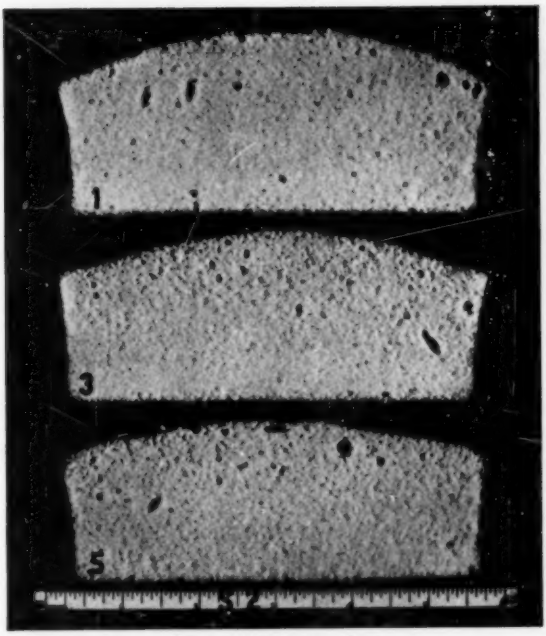

Fig. 2. Short patent flour.
Cake No. 1—Control
Cake No. 3—25% increase in shortening
Cake No. 5—50% increase in shortening

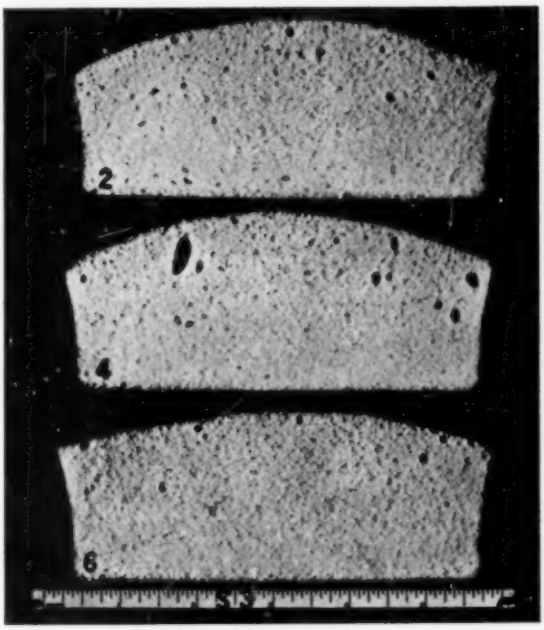

Fig. 3. Fancy clear flour.
Cake No. 2—Control
Cake No. 4—25% increase in shortening
Cake No. 6—50% increase in shortening

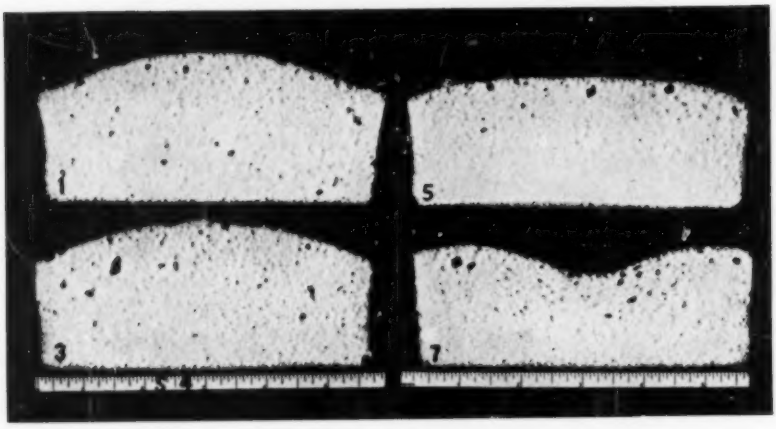

Fig. 4. Short patent flour.
Cake No. 1—Control
Cake No. 3—10% increase in sugar
Cake No. 5—20% increase in sugar
Cake No. 7—30% increase in sugar

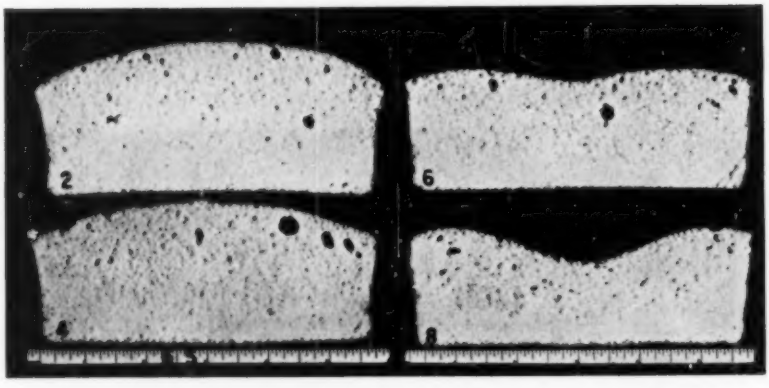

Fig. 5. Fancy clear flour.
Cake No. 2—Control
Cake No. 4—10% increase in sugar
Cake No. 6—20% increase in sugar
Cake No. 8—30% increase in sugar

less than 20%, and the short patent (Figure 4, cake No. 7) less than 30% increase.

The experimentally milled flour (Figure 1, cake No. 2) showed a shortening tolerance less than 25%; the fancy clear (Figure 3, cake No. 6) and short patent (Figure 2, cake No. 5) less than 50% increase.

With each increase in sugar the cakes became heavier, the grain coarser, the texture harsher, the crusts browner and flatter, until they finally fell.

With each increase in shortening the cakes became heavier and greasier, the grain closer, and the crusts flatter.

The decrease in volume and specific volume were apparent for each successive treatment (see Table II).

THE TECHNIQUE OF PHOTOGRAPHY FOR PERMANENT RECORDS OF BAKING STUDIES

LYDIA COOLEY and J. R. DAVIES

Calumet Baking Powder Company, Chicago, Illinois

(Read at the Annual Meeting, June 1935)

Do you remember the results of your experimental bakes, with whatever variables which were in test, of some five years ago? Are you able to describe from your mathematical scores the differences in grain, in symmetry, and in general appearances caused by variables in your studies of one year ago?

We believe that you are unable to do this with a fair degree of accuracy without photographs of your results. In our opinion, photographs are one of the best available means of permanently recording the results of baking research. Of course, a written record of physical and organoleptic measurements such as volume, specific volume, texture (response to touch or "feel"), taste or eating quality, and odor are essential.

Some years ago the necessity of a permanent record, other than the generally accepted scores, was forcibly brought home to us. Mr. C. G. Harrel's excellent paper on "Some Basic Principles of Photography as Applied to Cereal Work," presented at the Annual Meeting in 1930 (see Cereal Chem., 7: 313) was of inestimable value to us, and we also wish to acknowledge to Mr. Harrel our debt for his help, given in personal conversation.

We established a photographic department in connection with our research kitchen, a part of our chemical laboratory. We have used for many years an 8 x 10 copy camera. Generally speaking, our subjects are reproduced life or natural size; interior cross-sections, vertical and horizontal, as well as exteriors being photographed. We use commercial ortho film for most of our work. If one wants to show more clearly differences in color we suggest commercial panchromatic film.

A good lens is essential, but, as Mr. Harrel pointed out, "proper lighting is most important. The appearance in the positive of the

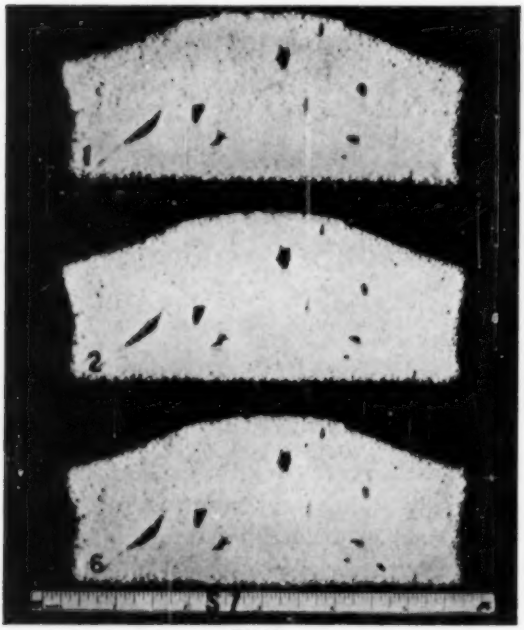

Fig. 1.

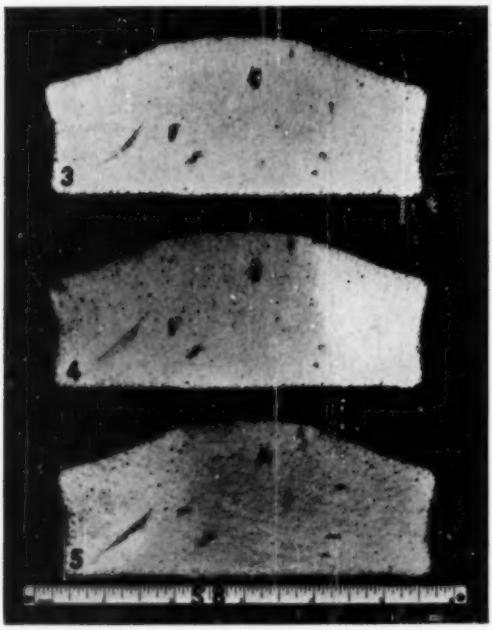

Fig. 2.

Figs. 1 and 2. Showing the effect of the angle of light—
In cake No. 1, the angle was 20°, and,
in cake No. 5, substantially 90°

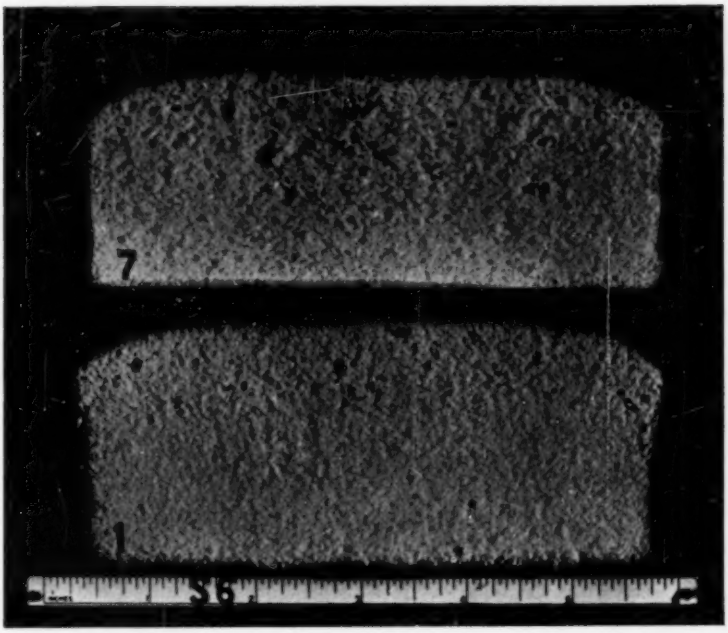

Fig. 3. Effect on characteristics of cake of varying amount of liquid.

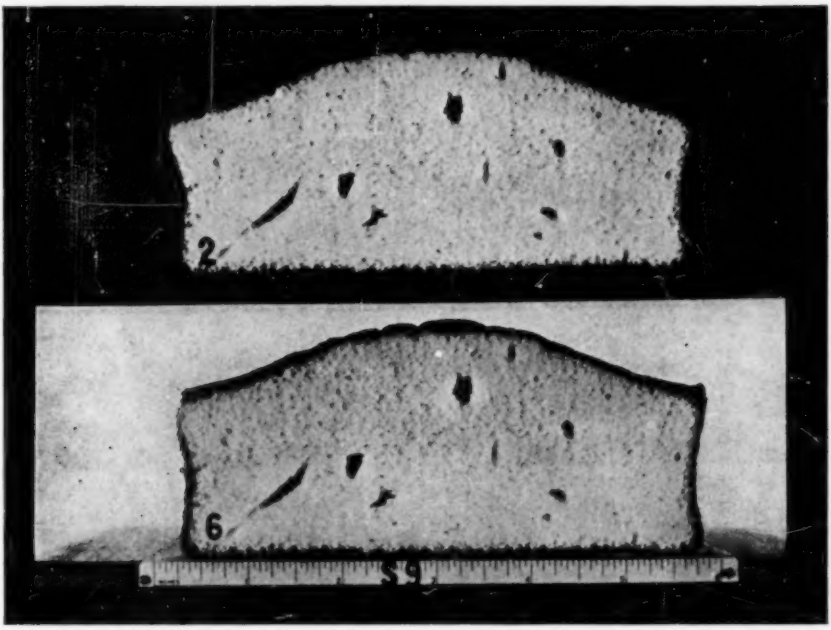

Fig. 4. Comparison of white and black background.

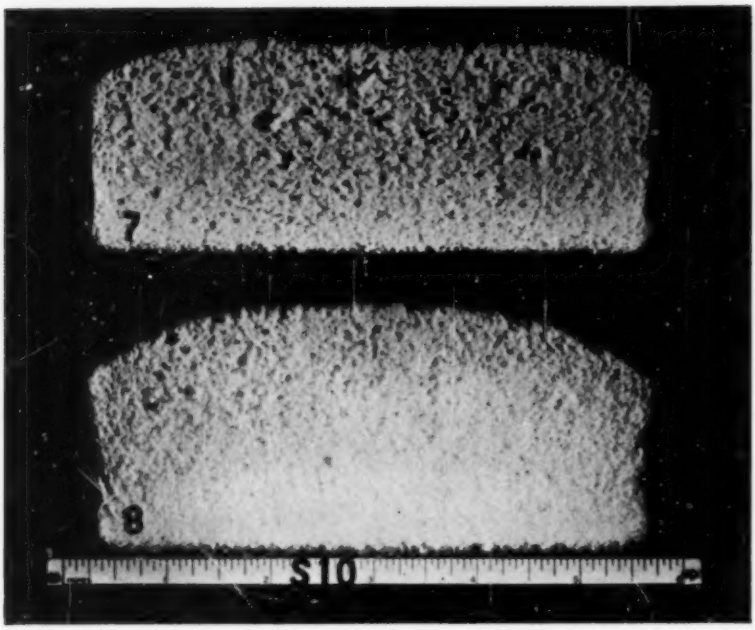

Fig. 5.

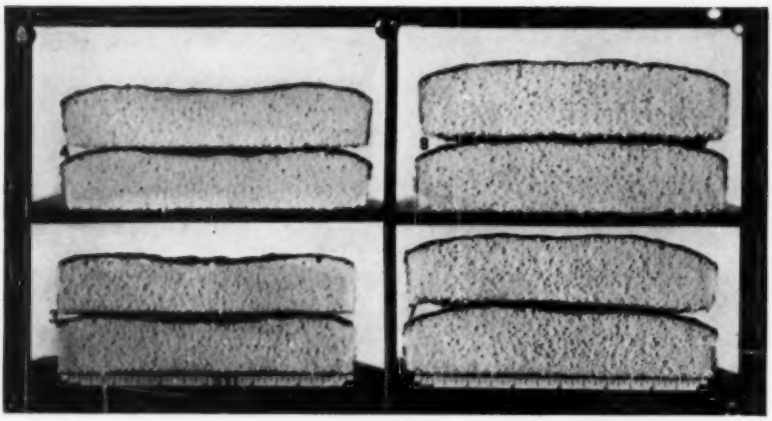

Fig. 6.

Figs. 5 and 6. Effect on characteristics of cake of varying mixing operation.

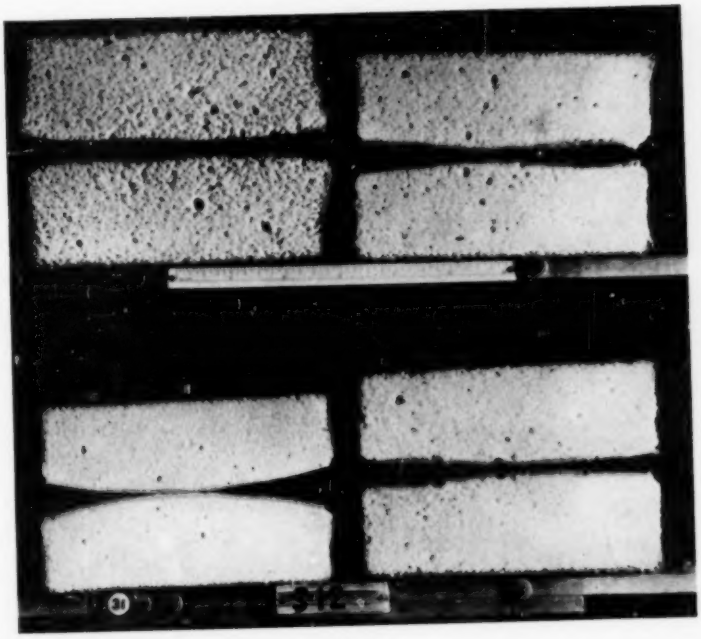

Fig. 7.

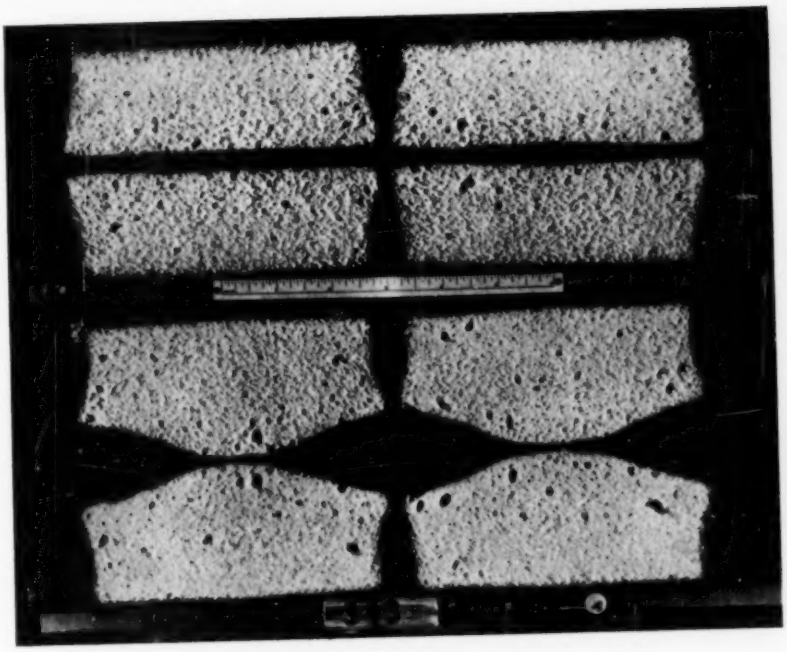

Fig. 8.

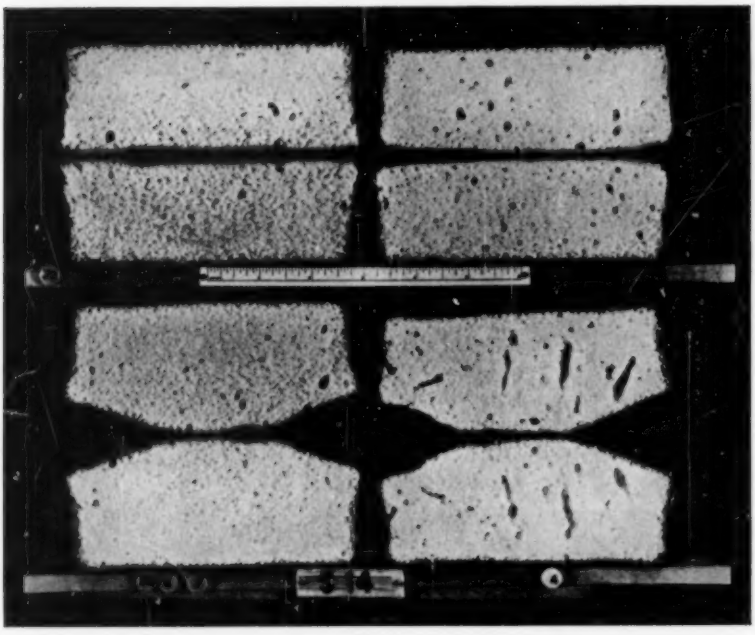

Fig. 9.

Figs. 7, 8, and 9. Effect on characteristics of cake of varying baking temperatures.

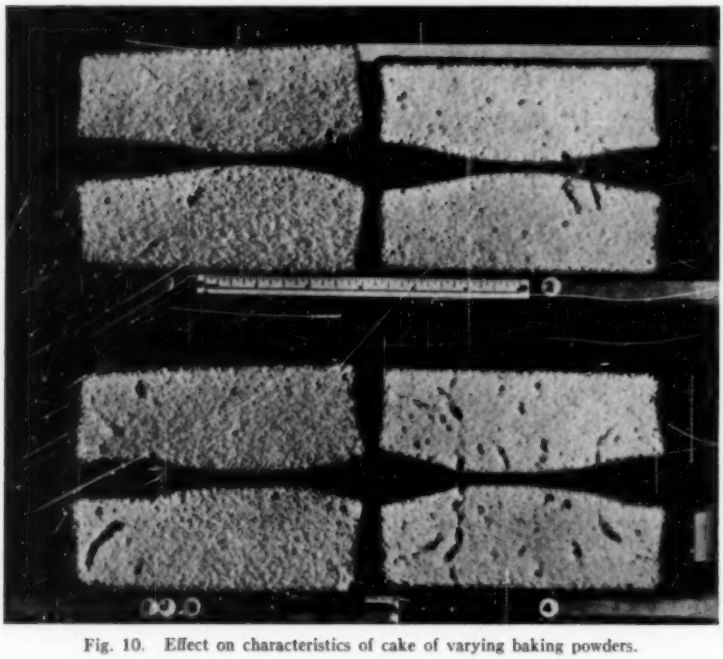

Fig. 10. Effect on characteristics of cake of varying baking powders.

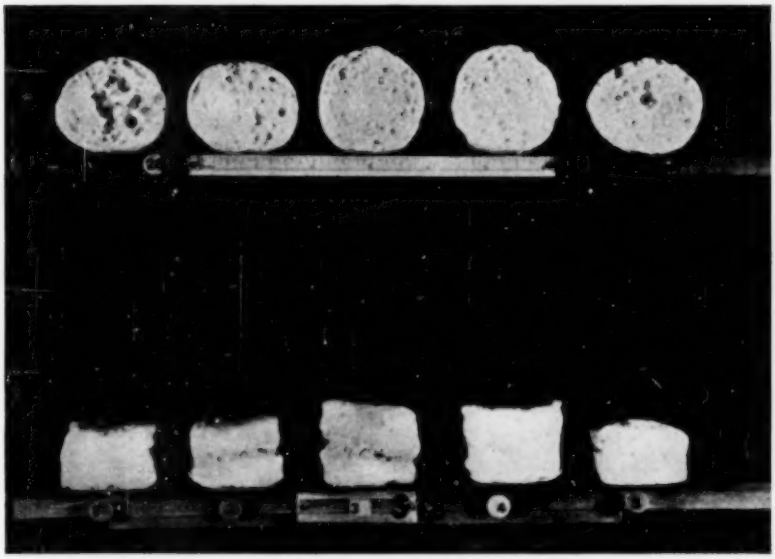

Fig. 11. Effect on characteristics of biscuits of varying baking powders.

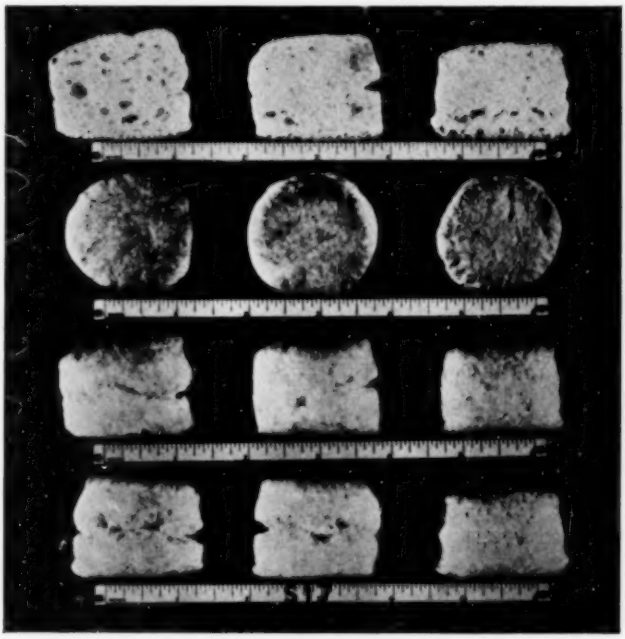

Fig. 12. Vertical cross-section, crust, front and rear view of biscuits.

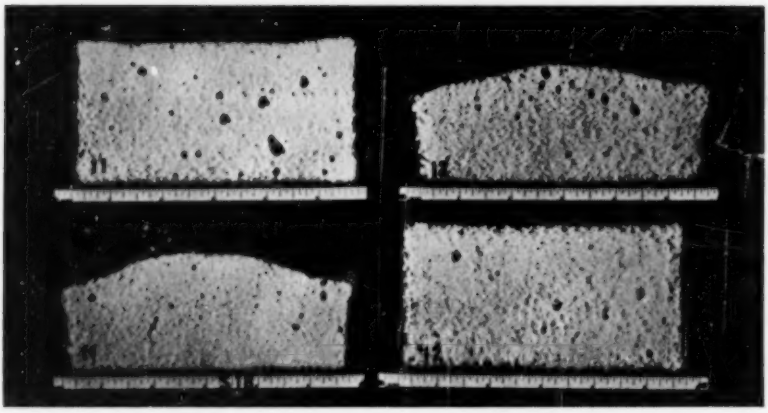

Fig. 13. Effect on characteristics of cake of varying quantity of baking powder.

grain . . . depends largely upon the angle of illumination." This angle of illumination is 20°.

In our routine procedure, we use two 400-watt photo 'flood-lamps, one on each side at 20° angle and 54 inches from the subject, with the camera, lamps, and subject in the same plane. The lens (Turner-Reich combination antistigmatic) is stopped down to 22, at which opening we generally expose for 35 seconds.

To show differences in crumb or grain we use a black background; to show up crust, we use a white ground.

Figures 1 and 2 illustrate the necessity of having the correct angle of light to bring out clear and true reproductions.

Figures 3–13 illustrate the clarity with which differences in baking results may be shown.

SUGAR TOLERANCE IN THE A. A. C. C. BASIC FORMULA FOR TESTING CAKE FLOURS[1]

W. E. STOKES and LAURA K. TRACK

Royal Baking Powder Company, Brooklyn, New York

(Read at the Annual Meeting, June 1935)

At a meeting of the A. A. C. C. Committee on Testing Cake Flours at Toronto in 1934, held to discuss tests for determining the effect of added sugar on the basic formula for testing cake flours, it was suggested by Dr. Glabau that to overcome certain variables the added sugar might be incorporated as syrup of known Baumé.

Dry Sugar vs. Syrup

SERIES A AND C

Tests were accordingly set up in one series "A" in which the original amount of sugar was increased by 10% increments added in dry form, and in another series "C" the increments were added in syrup of the same Baumé as would result if the original amount of sugar and the original amount of liquid in the basic formula were combined. The data from these tests are recorded in Table I. Cake characteristics other than volume are shown in Table VIII.

The control cake made up with all dry sugar as specified in the basic formula of the A. A. C. C., and the cake made with all syrup, were identical in volume. The grain of the cake made with the syrup, however, was a trifle more even, the color was clearer, and the texture slightly better than the dry-sugar cake (see Table I).

When a 10% increase in sugar was added to the basic amount of dry sugar in the cake formula, the dry-sugar increment produced a cake of approximately 5% larger volume than did the syrup increment. The total cake score was increased by the dry increment of sugar and decreased by the syrup increment. While a sharp falling off in volume, grain, and texture resulted from successive increments of syrup, the decrease in volume and general cake score was gradual with the addition of dry increments. The same average volume was obtained with the 30% increment of dry sugar as with the 10% increment of syrup.

While these tests show that adding the sugar in syrup form gave very marked decreases in volume, texture, and grain ratings, it is

[1] Subcommittee report, 1934–35 Committee on Cake Baking Tests.

believed that this was due to the increase in liquid in addition to that of the sugar because when sugar alone was added a 10% increase gave a cake larger in volume and better in texture. When syrup was added, the volume was smaller, texture closer and inclined to gumminess. The flour used in these tests had a tolerance to dry sugar equal to a 20 to 30% increase over the basic amount. Its tolerance to sugar in syrup form was only 10%.

TABLE I

TEN PER CENT INCREMENTS OF DRY SUGAR AND OF SYRUP

Series A and C

				10% increase		20% increase		30% increase		40% increase	
		250 g. Sugar	450 g. Syrup	275 g. Sugar	250 g. Sugar, 45 g. Syrup	300 g. Sugar	250 g. Sugar, 90 g. Syrup	325 g. Sugar	250 g. Sugar, 135 g. Syrup	350 g. Sugar	250 g. Sugar, 180 g. Syrup
Baking 1, volume, *Cc.*		824	824	863	798	796	720	777	694	774	486
" 2, " "		830	830	803	783	786	673	792	681	769	490
" 3, " "		—	—	807	775	—	—	786	638	—	—
Ideal Score											
External:											
Symmetry	10	9.5	9.5	9.75	9.5	9.5	6.0	7.5	5.0	5.0	0
Volume	15	15.0	15.0	17.0	14.0	14.0	10.0	13.0	8.5	13.0	0
Crust	5	3.5	3.75	4.5	4.25	3.0	3.0	2.75	2.5	2.0	0
Internal:											
Texture	30	25.0	25.5	26.0	24.0	21.0	15.0	20.0	15.0	20.0	0
Grain	25	22.0	23.0	24.0	22.0	22.0	11.0	20.0	10.0	20.0	0
Color	15	14.0	15.0	15.0	14.5	14.0	13.0	12.0	11.0	10.0	0
Total	100	89.0	91.75	96.25	88.25	83.5	58.0	75.25	52.0	70.0	0

All Syrup vs. All Sugar

SERIES B

Two series of cakes were made up in which were used all dry sugar in the basic and as increments, and all syrup in the basic and as increments. See Table II.

The basic cakes showed approximately the same results whether the sugar was used dry or in the syrup form. The 10% increment of syrup gave an approximately 6.8% smaller volume than the same increment of dry sugar; the 20% increment of syrup gave an approximately 17% smaller volume than the same increment of dry sugar; and the 30% increment of syrup gave an approximately 6.3% smaller volume than the same increment of dry sugar.

Adding the sugar to the mix in syrup form did not extend the tolerance point but did change the structure of the cake so that it was more dense and therefore did not collapse as under the featheriness of an increased dry-sugar content.

TABLE II

TEN PER CENT SUGAR ADDED DRY TO MIX. NO ADDITIONAL LIQUID. TEN PER CENT INCREMENTS OF SYRUP ADDED TO ORIGINAL AMOUNT OF 450 G., OR THE ORIGINAL AMOUNT OF SUGAR PLUS THE WATER IN THE BASIC FORMULA

Series B

		250 g. Sugar	450 g. Syrup	10% increase		20% increase		30% increase	
				275 g. Sugar	495 g. Syrup	300 g. Sugar	540 g. Syrup	325 g. Sugar	585 g. Syrup
Volume (1), *Cc.*		846	835	803	754	802	664	745	698
" (2), *Cc.*		848	832	819	758	804	670	—	—
Ideal Score									
External:									
Symmetry	10	9.5	9.5	10.0	10.0	9.5	7.0	5.0	7.0
Volume	15	18.0	17.5	16.0	13.5	16.0	9.0	13.0	11.0
Crust	5	4.5	4.5	5.0	4.75	4.5	3.0	2.5	2.75
Internal:									
Texture	30	30.0	30.0	28.0	26.0	27.0	20.0	20.0	22.0
Grain	25	22.0	23.0	20.0	21.0	19.0	18.0	12.5	20.0
Color	15	15.0	13.0	15.0	12.0	14.5	10.0	12.0	5.0
Total	100	99.0	97.5	94.0	87.25	90.5	67.0	65.0	67.75

Dry Sugar in 10% Increments with 10% Increments of Water. Syrup in 10% Increments

SERIES D

To determine whether or not any difference is reflected as between sugar and water increments added separately to the mix or added in syrup form, cakes were made up using the basic amount of dry sugar and the basic amount (450 g.) of syrup. The sugar and liquid were each increased by 10% increments and baked in comparison with cakes in which the syrup was increased by 10% increments. Thus, to the basic formula containing 250 g. of sugar and 200 g. of water were added 25 g. of sugar and 20 g. of water, etc.

To the basic formula made up with the syrup was added 45 g. of syrup bringing the amount to 495 g., etc.

The all-syrup batters were thicker and whiter than the sugar-water batters all through the series. Syrup cakes registered larger volumes than the sugar-water cakes. See Tables III and VIII.

Dry Sugar with Small Increments of Water

SERIES E

Believing that the all-syrup increments contained too much liquid for the structure of the cake, yet knowing that all sugar caused excess featheriness and collapse, a series of cakes was baked in which dry

TABLE III

SUGAR, DRY, WITH 10% INCREASES AND 10% INCREASES IN WATER. SUGAR IN SYRUP SOLUTION, 28° BAUMÉ WITH 10% INCREASES

Series D

	250 g. Sugar	450 g. Syrup, 20 g. Water	275 g. Sugar	495 g. Syrup, 40 g. Water	300 g. Sugar	540 g. Syrup, 60 g. Water	325 g. Sugar	585 g. Syrup, 80 g. Water	350 g. Sugar	630 g. Syrup
Volume, *Cc.*	822	822	746	758	689	740	695	722	675	681

sugar with dry increments was used, along with water at the rate of 10% upon the sugar increase. Thus, with the 10% sugar increase, or 25 g. of added sugar, 2.5 g. of water was added to the mix.

With this method collapse did not occur until the 40% increment of sugar was reached.

The 10% increment of sugar again registered best in volume and other characteristics, while the tolerance point might be placed at 30% (see Table IV).

TABLE IV

DRY SUGAR WITH INCREMENTS OF WATER

Series E

		250 g. Sugar	275 g. Sugar, 2.5 Water	300 g. Sugar, 5.0 Water	325 g. Sugar, 7.5 Water	350 g. Sugar, 10 Water	375 g. Sugar, 12.5 Water
Volume, *Cc.*		848	859 (1.3% increase)	835 (1.5% decrease)	824 (2.83% decrease)	784 (7.54% decrease)	731 (13.8% decrease)
Ideal Score							
External:							
Symmetry	10	10	10	8.5	8	7	0
Volume	15	15	15.5	14.5	14	12	9
Crust	5	4.5	4.75	4.25	.4	3.5	3
Internal:							
Texture	30	25	26	22	23	15	13
Grain	25	22	22	18	18	15	12
Color	15	15	15	14	13	10	9
Total Score	100	91.25	93.25	81.25	80	62.5	46

Sugar Dissolved in Liquid Original to Basic Formula

SERIES F

Apparently sugar in solution had marked effect upon volume and other characteristics. Therefore, cakes were baked in series, one series containing sugar added to the mix in dry form, the other containing sugar dissolved in the milk solution of the formula. Sugar in

both cases was increased by 10% increments. No additional liquid was used with additional sugar. With the lesser amounts of sugar there was small difference between the dry sugar cakes and the sugar-milk solution cakes except for progressively glossier crusts as the increments were added. With the larger amounts the sugar-milk syrup showed tendency to larger volume but not so even grain with a more marked collapse in the cakes containing the greatest amounts of sugar (Table V).

TABLE V

TEST BAKINGS ON A. A. C. C. FORMULA MADE TO DETERMINE DIFFERENCES BETWEEN CAKES MADE BY USING SUGAR IN DRY STATE AND THOSE MADE BY EMPLOYING SUGAR IN SYRUP FORM AS OBTAINED BY DISSOLVING THE DRY SUGAR IN THE MILK SOLUTION

Sugar, in both cases, increased by 10% increments. No additional liquid with additional sugar.

Series F

	250 g. sugar		275 g. sugar		300 g. sugar		325 g. sugar		350 g. sugar		375 g. sugar	
	Dry	In milk	Dry	In milk	Dry	In milk	Dry	In milk	Dry	In milk	Dry	In milk
Volume, *Cc.*	822	815	833	831	866	869	771	783	758	760	722	734

Effect of Increase in Leavening With Increase in Sugar

To determine the effect of leavening in these increased sugar mixes, cakes were made up with sugar-water increases and syrup increases. With each 10% increment in sugar or syrup, a step-up of 5% in leavening was used. Thus, with 10% increase in sugar, 5% increase in leavening, 20% increase in sugar, 10% in leavening, with the result that the limits of sugar tolerance in the syrup cakes was extended from 10% to 30%. The data with regard to the leavening studies are given in Table VI.

Indications from this test are that the limits of sugar tolerance may be extended by adding the sugar in the form of syrup of the same density as the original sugar-liquid combination in the mix when the leavening is also increased. While changing the form of the sugar and varying the methods of its incorporation into the mix produces marked effects upon volume, crust, grain, and texture, at the same time variables are introduced which may affect the apparent findings (Table VII).

TABLE VI

EFFECT OF INCREASE IN LEAVENING OVER ORIGINAL AMOUNT WITH INCREASES IN SUGAR-WATER AND SYRUP INCREMENTS OF 10%

Sugar and water increased by 10% increments	5%	10%	15%	20%
Syrup increased by 10% increments	6.3 3.15	6.6 3.3	6.9 3.45	7.2 Cream Tartar 3.6 Soda
	Sugar 275 g. Water 20 g.	Sugar 300 g. Water 40 g.	Sugar 325 g. Water 60 g.	Sugar 350 g. Water 80 g.
Volume, *cc.*	795	777	748	695
	Syrup 495 g.	Syrup 540 g.	Syrup 585 g.	Syrup 630 g.
Volume, *cc.*	774	760	772	722

TABLE VII

CAKE VOLUME SUMMARY; EFFECT OF DRY SUGAR AND SUGAR AS SYRUP IN A. A. C. C. BASIC CAKE FORMULA FOR TESTING CAKE FLOURS

Schedule of Average Volumes

Figures represent average volume of total number of bakings in each class.

Series Letter	Variables	Increases					
		Basic (250)	10%	20%	30%	40%	50%
A	Dry Sugar, volume, *cc.*	833	827	806	774	764	722
B	All Syrup, volume, *cc.*	829	756	740	772	700	—
C	Dry Sugar (basic amount), Syrup (increments), volume, *cc.*	833	777	698	675	525	—
D	Dry Sugar with increments of dry sugar. Increments of water equivalent to "C," volume, *cc.*	833	746	689	695	675	—
E	Dry sugar with water added at rate of 10% of sugar increase, volume, *cc.*	833	859	835	824	784	731
F	Sugar dissolved in milk. No increase in liquid, volume, *cc.*	815	831	809	783	760	734

TABLE VIII

EFFECT OF DRY SUGAR AND SUGAR AS SYRUP IN A. A. C. C. BASIC CAKE FORMULA FOR TESTING CAKE FLOURS

Series Letter	Treatment	Characteristics Other than Volume
A	Dry Sugar	Velvety to feathery texture up to 20% increase over amount in basic formula. Crust becomes successively darker, glossier, and tougher with each increase beyond 20%.
B	All Syrup	Sharper falling off in quality as to general appearance, crust, texture, etc., with increases of dry sugar. Crust becomes successively stickier beyond 10% increase although the tolerance point could be placed at 20%. Texture runs from feathery to gummy; color becomes more gray as more syrup is used.
C	Dry Sugar (basic amounts) Syrup (increments)	More solid structure than dry sugar cakes becoming closer with each increment of syrup whereas dry sugar becomes fluffier with each increment to the point of collapse.
D	Dry Sugar with increments of dry sugar and increments of water	Cakes under "C" slightly topped "D" on all counts.
E	Dry Sugar with water added at rate of 10% of sugar increase	Cakes in this test showed a slight tendency to better structure under increased sugar content but here again the tolerance was approximately 20%.
F	Sugar dissolved in milk	With the lesser amounts of sugar there was small difference between the dry sugar and the sugar-milk cakes, except for progressively glossier crusts of the latter. With the larger amounts the sugar-milk syrup showed tendency to larger volume but not so even grain and a more marked collapse in the cakes containing the greatest amounts of sugar.

Conclusion

In our opinion, for the purpose of determining the sugar tolerance of a flour, the use of dry sugar without any variable as to method of adding the increments to the amount of sugar in the basic formula is to be recommended.

BOOK REVIEWS

Das Problem der Backfähigkeit. By Natalie P. Kozmin, Professor of Cereal Chemistry at the Institute of Milling Technology, Tomsk, U.S.S.R. Published by Moritz Schäfer Verlag, Leipzig, 1936. 195 pages, 39 illustrations, 74 tables, parchment binding. Price (including postage and discount to foreign purchasers) 6.23 Reichsmarks.

This book by the eminent Russian cereal chemist represents the first adequate outline of the newer developments in the biochemistry of the cereals, with special reference to the baking process, to appear in several years. The author's aim has been "to investigate the problem of flour quality from the biochemical point of view, on the basis of a study of the entire process of bread making." That particular attention has been paid to the more recent literature in this field is evidenced by the fact that approximately three-fourths of the papers covered in the book have been published during the last ten years. The contributions of American cereal chemists represent more than half the total number of papers listed in the bibliography.

The important studies of the author and her co-workers on the aging of wheat flour, carbon dioxide production and retention, the relation between diastatic activity and baking quality, the effect of various factors on gluten quality, the colloidal nature of wheat gluten, the gliadin-glutenin ratio and numerous other subjects are discussed at some length.

The introductory chapter presents a short survey of the entire technological process of bread making, in which the baking process is described in terms of the changes in the physical and chemical properties of the proteins and carbohydrates of the dough. Chapter I deals with the enzymes of flour. The various methods of determining diastatic activity are outlined and compared, and the effects of temperature, pH, wheat variety and character, starch characteristics and sprouting on diastatic activity are described. The proteolytic and other enzymes are treated rather briefly.

The formation of dough is the subject of Chapter II. Under this head are included the structure of wheat flour dough, gluten formation and factors affecting this process, and the effects of electrolytes, fats and fatty acids, oxidizing agents, temperature and physical treatment on gluten quality. A few of the devices for testing the mechanical properties of gluten and dough are described in this chapter. Some interesting suggestions for research are made in connection with the discussion of the properties of rye flour dough.

Chemical changes in dough during fermentation and the accompanying changes in physical structure are covered in Chapter III, which takes up production of fermentable sugars, loss of carbon dioxide, the relation between amount of yeast and gas production, changes in acidity, and chemical and physical alteration of the proteins.

Chapter IV, on the baking of the dough, shows how the chemical composition of the dough is changed during the baking process. Of particular interest are the figures showing the composition of the water-soluble fractions of dough and bread from flours of high and low diastatic activity.

The principal processes of bread making are briefly characterized in Chapter V.

Chapter VI is devoted to bread from defective flour and methods for its improvement. The chemical and physical properties of bread from flours milled from wheats of varying diastatic activity are set forth in considerable detail, and the results obtained by the author in correcting excessive diastatic activity through lowering the pH of the dough to 4.3–4.5 are presented. The cause and nature of "slimy gluten" in insect-damaged wheats are discussed, and the effect of lowered pH and the addition of free unsaturated fatty acids upon doughs made from flour with such gluten are shown.

Methods for testing the baking quality of flour are outlined in Chapter VII. It is interesting to find here a revaluation of the pioneer work of Humphries, Wood and Saunders, and a detailed account of the baking method and bread-scoring system used by the last-named investigator. A number of pages are devoted to a

description of the A.A.C.C. Baking Test and an account of the work of the Baking Test Committee. Dr. Kozmin states that Europe is considerably behind America in regard to the baking test; many different methods are in use in the mill and bakery laboratories, and the results are not mutually comparable. A few of these methods are described, although only very briefly. It is rather surprising to find no mention made of the work done by Dr. Kent-Jones in this field. In discussing the correlations between protein content and loaf volume by various baking procedures, the author expresses the opinion that American investigators have laid too much stress on loaf volume, and have not sufficiently considered the important effect of protein content on other baking characteristics, notably water absorption.

Chapter VIII, on flour improvers, is shorter than one might anticipate, in view of the variety of improvers on the European market and their widespread application in certain countries. Malt flours and malt extracts, gelatinized flours, inorganic salts and heat treatment are briefly discussed.

The book is well organized, and the presentation of the material is clear and concise throughout. It is unfortunate that neither a subject nor an author index has been included.

CLINTON L. BROOKE

The Chemists' Year Book (1936), 18th Edition. Edited by E. Hope, Fellow of Magdalen College, Oxford. Published by Sherratt & Hughes, Manchester, England. Agents for North and South America, Chemical Publishing Company, New York, N. Y. 1237 pages and complete index, $3\frac{1}{2} \times 4\frac{1}{2}$ inches. Price $6.00.

This new and revised edition, the compilation of over 25 collaborators, is a veritable storehouse of information for the industrial chemist. The subject-matter is divided into 57 sections and subsections.

The first 500 pages are devoted largely to tabular matter of the following order: atomic weights, physico-chemical constants, solubility tables, tables of refractive indices, dissociation constants, properties of minerals, general properties of inorganic compounds, nomenclature, and general properties of organic compounds (to which section there have been extensive additions). A chapter on crystallography is also included.

600 of the succeeding pages are devoted to comprehensive directions relative to analytical procedures of various sorts, such as electrochemical gas, qualitative, spectrum, volumetric, and pH analyses, as well as specific chapters devoted to the analysis of special materials such as acids and alkalies, alkaloids, brewing materials, carbohydrates, cellulose and paper, coal tar, cement, dairy products, dye stuffs, essential oils, feeds, fertilizers, fuels and illuminants, leather, lubricants, manures, oils, fats and soaps, paints and pigments, physiological products (urine, blood), refractives, rubber, soils, spirits, tanning materials, textile fibers, tobacco, and water.

A new section is included in this edition on the analysis of sulphuric acid, treated oils, alcohols, and wetting agents.

The remainder of the book contains a chapter on patent law and chemical invention, a glossary of medical terms, hydrometric tables, tables for the interconversion of thermometric scales, a 5-place logarithms table, etc.

The tabular matter in the book is universal in its application. On the other hand, the analytical procedures are largely British, although in a few instances comparable American methods are given.

For the cereal chemist, analytical methods related to the cereals and cereal products, except for the analysis of feeding stuffs, either of American or British origin, are absent. Nevertheless, the book is well edited and is of value to chemists in all fields of industrial activity as a ready-reference volume in which is listed many types of chemical analyses as well as valuable reference tables.

D. A. COLEMAN

Yearbook of the United States Department of Agriculture—1936, and Agricultural Statistics—1936. For sale by the Superintendent of Documents, Washington, D. C. Price $1.25 and 50¢ per volume, respectively.

The 1936 Yearbook of Agriculture differs in two ways from previous years editions, in that the agricultural statistics, which heretofore have been a part of the yearbook, have this year been pblished separately, and the bulk of the yearbook is devoted to a single subject—the creative development of new forms of life through

plant and animal breeding, rather than the brief summaries of miscellaneous new developments in agriculture formerly carried. These special articles are the outcome of a survey of superior germ plasm made by the Committee on Genetics. What this superior germ plasm is and how it is used constitute a story of surpassing importance to the modern world.

Of particular interest to the cereal chemist are the following articles: Improvement in Wheat, Problems and Results in Barley Breeding, Superior Germ Plasm in Oats, Improvement in Rice, Corn Improvement, and Sorghum Improvement.

D. A. COLEMAN

Flour-Mill Insects and Their Control. U. S. Dept. Agr. Circ. 390. By G. A. Dean, Kansas Agricultural Experiment Station, and R. T. Cotton and G. B. Wagner, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. For sale by the Superintendent of Documents, Washington, D. C. Price 5¢.

Of timely interest to all those concerned with flour mill operation is this recent circular issued in July. The insect pests of stored grain found on the farm as well as in grain elevators, insect pests of flour mills, and their avenues of infestation are discussed in detail. Control of insect infestation by sanitation as well as by different types of fumigation is described. A section is also devoted to the control and elimination of insects in the warehouse and of flour in transit. Instructions are given for control of insects in returned goods and used bags. The various types of chemical means of fumigation as well as means of fumigation by heat are covered in this exhaustive discussion of flour-mill insects and their control.

D. A. COLEMAN

Presentation

of the

Thomas Burr Osborne Medal

to

Morris J. Blish

by the

American Association of Cereal Chemists

June 2, 1936

MORRIS J. BLISH

REPORT OF THE THOMAS BURR OSBORNE MEDAL AWARD COMMITTEE

PAUL LOGUE, *Chairman*

I have attended eleven consecutive annual conventions of the American Association of Cereal Chemists. There is a singularly close relationship between the first of these in Denver and the present one here in Dallas. It was in Denver in 1926 where the natural inclinations of scientists to render honor to their fellow-workers, who distinguished themselves with outstanding efforts, took definite form in a proposal by President Clark in his address to the convention. This proposal received favorable consideration by the Executive Committee resulting in the adoption as a policy of the Association of the periodic award of a suitable medal for distinguished service in Cereal Chemistry.

A committee was selected, chairmaned by Dr. Carl L. Alsberg, of which committee I was honored to be a member, charged with the duty of setting forth the rules governing the basis of such awards, the selection of a medal, and of a medalist. The committee chose as a first recipient of the medal Dr. Thomas Burr Osborne, and on request Dr. Osborne very graciously granted the Association the privilege of naming the medal the Thomas Burr Osborne medal. The medal, a gold one, bears on its obverse side a portrait of Dr. Osborne in low relief and on the reverse is inscribed the medalist's name.

Dr. Osborne, as many of you know, devoted a lifetime of study to vegetable proteins and related subjects at the Connecticut Agricultural Experiment Station. In his earlier years he received so little recognition for his work that he refrained from publishing the results of his carefully planned and scientifically executed investigations in the reports of the Connecticut Agricultural Experiment Station lest when questions of an appropriation should arise members of the Legislature should ridicule his work as of no practical value to the farmer. It remained for the Germans to discover and to show proper appreciation of the scientific merit of his great work and even

in this country it was biologists and physicians who first gave him the recognition that was his due rather than those who were interested in the chemistry of cereals. If there are among you those who feel that your work goes unappreciated it would be a source of encouragement and inspiration to read the simple letter of acceptance written by Dr. Osborne from his sick bed, and read by Dr. Alsberg on the presentation of the medal. It is gratifying to me, and I feel that the whole Association shares in this view, that we were able to confer on him this honor during his lifetime, even though it reached him only when the evening shadows were lengthening.

The Thomas Burr Osborne Medal is awarded by the American Association of Cereal Chemists for distinguished contributions to cereal chemistry. The presentation on this occasion—the third since initiated in 1926—is to Dr. Morris J. Blish in recognition of his valuable and painstaking work on the proteins of wheat.

The first medalist was a man who had devoted his entire life to fundamental research. The second was one of a more varied experience—a man who has lived among us, and distinguished himself as an educator of cereal chemists—as a researcher in the field of cereal chemistry, and as director of research in one of the largest commercial firms dealing with cereals. We are fortunate tonight that we have him with us and that, as one medalist discussing the work of another, we may hear him address us on "The Work of Morris J. Blish." I present to you Dr. Clyde H. Bailey.

THE WORK OF MORRIS J. BLISH

C. H. Bailey

In the late eighties of the last century Frank M. Blish and his wife Louise Joslin Blish moved from Illinois to Lincoln, Nebraska where the former became manager of the local office of R. G. Dunn and Co. Here on April 21, 1889 was born their son, Morris Joslin Blish. The Blish family continued in Lincoln for several years, where the father was active in civic affairs as a member of the newly organized Chamber of Commerce and the school board. He was also interested in outdoor sports, and fond of good literature and music; traits which were transferred to the son either by inheritance or precept.

In 1902 the family moved to Omaha, Nebraska, where the son Morris attended grade and high school. He then decided that he had had enough of formal schooling, and went to work for a large Omaha bank. After a year of such activities he suddenly discovered that the bank management was about to promote him to the position of bookkeeper. This filled him with dismay and he promptly decided that of the two evils he would prefer to undertake a University course rather than to become an accountant. It then became necessary to select a curriculum and while the son's leanings were largely toward music, the father objected to a professional musical career, and suggested the field of civil engineering. It was not long, however, before the student's interests became more and more centered in chemistry, and in due course the Bachelor of Science degree was earned by him in the Arts College of the University of Nebraska. This was in 1912. Blish then entered the Graduate School of that University and researched on soils, receiving the Master of Science degree in 1913.

At about this time two notable scientists were being recruited by the University of Minnesota in the persons of Dr. F. J. Alway of the University of Nebraska, and R. W. Thatcher, a Nebraskan who was then director of the Washington Agricultural Experiment Station. It was under Dr. Alway that Blish had conducted his researches at Nebraska, and there he had also become acquainted with Professor Thatcher. In selecting an institution in which to pursue further graduate studies Blish naturally turned to Minnesota where he could continue his relations with these two scientists. Here he entered

upon a program of study which involved the application of new techniques, including the Van Slyke method to the study of the chemical constitution of flour proteins. Thus was instituted a line of research which the medalist continued through the intervening years and with very fruitful results.

Professor Thatcher proved to be a dynamic leader with a tremendous capacity for both executive and scientific work. He was never terrified by new problems, and swept his younger associates

Dr. Blish Develops a Diastatic Activity Method

along by his enthusiastic and forceful leadership. I am certain that Dr. Blish will agree with me that those were joyful years. Apparatus was none too plentiful and often had to be devised and constructed on the job. Quarters were cramped, space was crowded, and we were forced to be content with improvised laboratories in what were originally the store-room and the shop of the old chemistry building. Despite these difficulties steady progress was made in advancing our knowledge of the flour proteins.

Naturally set-backs occurred occasionally and sometimes in the most surprising ways. Thus on one occasion Blish had several gliadin preparations, on which he already had spent many weary weeks in isolation and purification, and which were now in the final stages of drying in a vacuum oven. This oven was connected to an old style Chamberlain vacuum pump through which a flowing stream of water was presumed to supply the requisite aspiration. For some reason or other, the plumbers decided to turn off the supply of water, and this without warning anyone in our laboratory. As those who have had experience with such pumps are painfully aware, on stopping the normal flow of water, the vacuum system promptly relieved itself by sucking water back into the vacuum chamber. Before Blish could get to the hose connections, there was a rush of tap water in upon his precious gliadin, and a few seconds later, on opening the oven chamber, there was the sticky stuff plastered over its inner wall, and utterly ruined for scientific purposes.

Again at a later time he had a quantity of the same protein in alcoholic solution evaporating under vacuum in one of the few Claissen flasks owned by the department. Evidently the glassblower had not built the walls of the flask heavily enough to stand the hundreds of pounds of pressure exerted upon it under a high vacuum. At any rate, when the condensation was half completed the flask collapsed with an explosive roar, and the Norwegian janitor, Blish, and I were thoroughly deluged with alcoholic gliadin solution. Hair, exposed epidermis, garments all received their quota. A new scientific demonstration of the adhesiveness of this protein was immediately in progress. Ordinary glue could not possibly produce the sensation of evaporating alcoholic gliadin solution in one's hair and on one's epidermis. Blish and I possibly were more willing scientific martyrs than the Scandinavian janitor who cursed us roundly for such rough and unpleasant treatment.

Despite these and other difficulties Blish's researches moved steadily forward, and in 1915 the highest degree granted by American Universities, Doctor of Philosophy, was conferred upon him by the University of Minnesota. Incidentally he was the first candidate for this degree from the newly organized Division of Agricultural Biochemistry, of which Professor Thatcher was chief.

Blish then undertook a special study for the Anaconda Copper Co., with laboratory headquarters at Bozeman, Montana. Early in 1916

he became connected with the U. S. Bureau of Chemistry for a few months but soon returned to Montana as a member of the chemical staff of the Agricultural Experiment Station. Here he instituted researches in cereal chemistry, with particular reference to the general problem of frost damage in wheat. Several papers were published which contributed substantially to our understanding of the biochemistry of premature freezing, and the chemical characteristics of frosted grain. In addition he worked out the factors which determine the quality and composition of sunflower silage, and the digestibility of such forage, a matter of considerable importance in the higher altitudes where corn cannot be depended upon as a silage crop.

Dr. Blish Engaged in Sugar Analysis

While in Bozeman, Blish met the lady who later became his wife, Vera Buell, daughter of a business man of that city. Consequently the Blish family have continued their interest in Bozeman and its environs, and have spent many holiday periods in the Mountain State.

War service interrupted the course of his researches in Bozeman, but after a period of training, and a tour of duty in France, Blish returned to Bozeman early in 1919, and continued his work there until 1922 when he was appointed to his present position as head of

the Department of Agricultural Chemistry at the University of Nebraska.

Again Blish found himself in an area that has a major interest in cereal production and the cereal industries. This afforded an added opportunity for researches in the field in which he was already well established, an opportunity which he promptly embraced. A steady flow of papers concerning the wheat proteins now appeared from the Nebraska laboratory. A method for the direct determination of glutenin was devised. Effects of various treatments upon the properties of the proteins were indicated, and it was emphasized that alkaline treatments should be avoided. Later it was established that two proteins could be precipitated from an alcoholic solution of gluten containing an equivalent of 0.05 N acetic acid and a small amount of K_2SO_4. A protein resembling glutenin came down at about 19° C., while another portion was precipitated on cooling to 12° C. The latter, termed mesonin, is being studied further by Blish and other workers.

The various phenomena which collectively determine the "gassing rate" of dough also came to be a subject of major interest in the Nebraska laboratory. Blish and his colleagues called attention to the virtues of the ferricyanide reduction method that two Danes, Hagedorn and Jensen, had previously devised for determining blood sugar. This method was adapted to a modification of the Rumsey technique for estimating diastatic activity of flour, and is proving exceedingly useful and satisfactory.

In the same connection these workers have devised an instrument for measuring gassing rate in a direct manner by observing the pressure developed in a flour preparation fermented in a standard manner.

Certainly the cereal industries owe a major debt to Blish for his studies of the experimental baking test. In his laboratory the known factors or variables involved in the standard test, including dough ingredients, mechanical equipment, baking facilities and loaf scoring have been studied in detail. The American Association of Cereal Chemists selected his department in which to locate its research fellow for the study of many of these variables. Blish has held steadfastly to the fundamental thesis that a baking test to be a standard test must first be completely standardized and then followed meticulously. Also that a truly objective approach to the application of baking tests in flour appraisal must involve a measuring and scoring system which

is separated from the operator's preferences, whims and prejudices. A gradual recognition of the force of these contentions is moving the standard baking test into a more dignified and rational position in measuring and recording the baking characteristics of flour.

In addition to these studies of the cereals, Blish has attained also distinction for his researches on the metabolism of poultry.

He has served as an officer of the Nebraska Section of the American Chemical Society, and of the Graduate Council of the University of Nebraska. He is a member of the professional chemical fraternity Alpha Chi Sigma, the honorary chemical society Phi Lambda Upsilon, the honor society in agriculture Gamma Sigma Delta, the scholastic honor society Phi Kappa Phi, and the Society of Sigma Xi.

We remember him as President of this Association during the two years of 1923–25. During that period I worked with him very closely in launching the journal Cereal Chemistry, and in cementing the relations between the American Association of Cereal Chemists and the American Society of Milling and Baking Technology which had just amalgamated. This was a large task, which Blish handled in masterly fashion, and those of us who served with him had our respect enhanced by his manifest executive capacities.

While that was a substantial and strenuous job, I suspect that Blish found his duties as chairman of the committee on baking tests even more trying. This called for all the force and diplomacy of which a man is capable. Again, as in his other offices, Blish discharged his responsibilities in a fashion which greatly advanced this important project.

These activities should not be recorded wholly in the past tense, for it is clearly obvious that the man whom we are honoring with the Osborne Medal tonight is vigorously active during the current period. He is always alert to new developments in a wide range of scientific fields; he always has new problems to discuss; he is frankly and keenly critical of inadequately supported hypotheses and ready to defend his views. These are characteristics which mark the active and contributing scientist; characteristics in which we find satisfaction, which mark our progress; characteristics which we delight to honor.

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DR. BLISH AT HIS DESK

ADDRESS OF THE PRESIDENT OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS AT THE PRESENTATION OF THE THOMAS BURR OSBORNE MEDAL

WASHINGTON PLATT

A man can serve the profession of cereal chemistry in three separate ways. First, he can fill the position in which he is placed, whether in an educational or an industrial organization, in such a manner as to reflect credit on this department of his organization. Second, he can advance the science of cereal chemistry by his researches. Finally, he can assist in the work of our Association by service on our committees or as an officer of the Association. It is a remarkable fact that you, Dr. Blish, have rendered distinguished service in all three fields of cereal chemical activity.

It gives me great pleasure, both officially and personally, to present you with the Thomas Burr Osborne gold medal in behalf of the American Association of Cereal Chemists.

ACCEPTANCE OF THE OSBORNE MEDAL BY M. J. BLISH

Mr. President, Mr. Chairman, Friends:

I think I clearly recognize the fortuitous circumstances that have combined to make this award possible. My various activities and endeavors as a cereal chemist have always been very largely governed by favorable environments, associations, contacts, privileges and assignments. To all of these circumstances, individually and collectively, I make full and grateful acknowledgment. A very large share of this medal should rightfully belong to my friend and coworker during the past 14 years, R. M. Sandstedt.

I assure you, Mr. President, of my most sincere appreciation for this evidence of good will extended to me by yourself, and by my fellow-chemists whom you represent.

It is an added pleasure and distinction to have as my spokesman and "Counsel for the Defense" upon this occasion Clyde H. Bailey, himself the only other living Osborne Medalist, who gave me my first real introduction to cereal chemistry some 23 years ago, and who has been a friend and an inspiration during the entire period of our mutual acquaintance.

I can perhaps best indicate my general reaction to this event by using some language borrowed from that eminent social scientist, the late Will Rogers: "What you folks are saying about me ain't true, but I love to hear you say it."

Thank you.

THE WHEAT FLOUR PROTEINS

M. J. Blish

There are at least two reasons why I consider it appropriate, upon this occasion, to speak briefly about the wheat flour proteins. First, it is largely because of Osborne's notable contributions to our knowledge of these and similar substances that he has deservedly been accorded conspicuous honor and distinction, not only by the cereal chemists as a specialized group, but by biochemists as a whole. Secondly, in our Nebraska Agricultural Experiment Station laboratories, during the past 10 years, much time, effort and funds have been devoted to flour protein studies. This work has been far more extensive than might be suspected from the fact that very little of it has as yet been reported in detailed published form. It seems that as we have become increasingly familiar with the behavior and properties of flour proteins, we have found ourselves progressively more and more hesitant in the matter of making dogmatic assertions and published statements relative to their nature, their identities, and their individualities. It is this situation that has been chiefly responsible for our failure, thus far, to publish our findings in any considerable detail. The fact is that one must work with these proteins for years in order properly to appreciate the difficulties and complexities involved.

During the quarter of a century from approximately 1900–1925, it was generally supposed that the identities and the individualities, respectively, of the flour proteins were definitely established, largely as a result of Osborne's researches. During that period, gluten, the distinctive structural component of bread dough—supposedly constituting about 80–85% of the total flour protein—was believed to consist essentially of a mixture of two individual proteins, gliadin and glutenin. The remaining 15–20%, known as the non-gluten or "soluble" protein, was thought to be composed largely of the two individual proteins leucosin and edestin.

Since 1925, or thereabouts, as the result of researches conducted in the laboratories of Sorensen, Svedberg, and others, we have been

compelled gradually to abandon our time-honored belief in the chemical individualities of many, if not most of the various protein substances that have been isolated from biolgical material, named and classified. Casein was one of the first proteins to be reversibly fractionated, and found to be a mixture of components, not a homogeneous substance. More recently the individualities of other proteins have been seriously challenged, and among these are the proteins of wheat, especially the so-called gluten proteins.

As a result of our own work, confirmed by others, we now realize that "glutenin," as prepared according to Osborne's directions, is not a simple, unaltered, homogeneous protein substance, but is a derived protein that has undergone irreversible and destructive alteration as a result of exposure to the alkali used in its preparation. Svedberg and his associates have found both glutenin and gliadin to be heterogeneous substances, while Haugaard and Johnson report that they have fractioned gliadin, thereby proving its non-individuality. Gortner and associates find the globulin of flour to be an indefinite substance.

Sorensen considers that all soluble proteins are "dissociable, reversible component systems." Sandstedt and Blish have indicated their belief that the gluten proteins fall somewhat into this category, but that it is possible to distinguish three main component groups. McCalla and Rose also believe that Sorensen's concept applies to the gluten proteins. However, they found no basis for distinguishing special groups, and they conclude that gluten is "a single protein complex which may be separated into a great many fractions which differ progressively and systematically in both physical and chemical properties."

It is not my intention, upon this occasion, to argue or to insist that either of these viewpoints is the correct one. Our experiments have yielded two types of evidence, one type supporting the belief of McCalla and Rose that there are innumerable components differing "systematically and progressively," and the other type supporting the idea that the gluten components occur as distinct groups or individuals. This anomalous situation means, of course, that either there have been faults or errors in some of our methods of obtaining evidence, or that there has been some misinterpretation of existing evidence, or both. Either of these contingencies is always possible, if not probable, in research involving protein isolation and fractionation.

The procedure most commonly used for studying protein individuality is that of fractional precipitation. So long as successive fractions show a constancy in physical properties, and in chemical composition, there is a strong presumption that the substance under examination is a homogeneous chemical entity. But, as Osborne stated, when these successive fractions differ in their physical and chemical properties, there is no doubt that the substance in question is a mixture.

At first thought it might be judged a relatively simple matter to apply these criteria to the flour proteins, but in actual practice serious obstacles are encountered, both in the establishment of a trustworthy experimental technique, and in the interpretation of results. Much of our trouble and uncertainty can be traced to the dispersing agents that have been used to "extract" or disperse the protein material itself, which is obviously an essential preliminary step in fractional precipitation.

The ideal dispersing agent would be one that dissolves the flour protein selectively, that can be filtered or centrifuged to remove all foreign suspended material, and from which all of the protein can be recovered, either as a whole or in fractions, without loss or irreversible change in original properties. No solvent has been discovered or recognized as meeting all of these requirements, and this in itself has been one of the chief handicaps to progress in any efforts to establish the true nature of gluten. Many investigations have failed to take into account hydrolytic or other changes induced by the solvent itself.

Cook and Rose have rendered a real service by making a critical study of the extent to which protein hydrolysis occurs when gluten is dispersed in various solvents, including dilute alkali, dilute acid, and the two neutral solvents, urea and sodium salicylate. Combining the observations of Cook and Rose with our own findings, the following conclusions seem warranted:

Irreversible and destructive hydrolytic changes occur almost at once, and proceed at a relatively high rate when gluten is dispersed in dilute alkali. Alkaline dispersing media are therefore entirely unsuitable for gluten fractionation studies. In dilute acetic acid, an appreciable amount of hydrolysis at room temperature is detectable after a few hours' standing, but this is more a result of enzyme action than an effect of the acid itself, as is shown by the fact that it proceeds more slowly in N/1 acetic acid than in N/10 acetic acid. When dispersion in dilute acetic acid is carried out at ice temperature, the dis-

persion can be kept for several days at this temperature without undergoing significant hydrolytic change. One great advantage of dilute acetic acid dispersions is that all starch can be removed by means of a Sharples supercentrifuge operating at approximately 40,000 revolutions per minute.

The addition of an equal volume of alcohol to a dilute acetic acid dispersion of gluten is highly protective against hydrolytic change, and we have used this procedure in many of our fractionation studies. Some recent findings in our laboratory, however, have disclosed the fact that alcohol itself affects what seems to be the irreversible splitting of an important gluten component, namely, a "lecitho-protein" or some similar lipoid-protein complex. We suspect that this complex is a very important gluten constituent, and that both its importance and the amounts in which it occurs have heretofore been generally underestimated. This lipoid-protein complex is apparently unaffected by ether, but it seems increasingly probable that its disruption by alcohol is responsible for profound changes in the solubilities and in the colloidal properties of some of the essential gluten components.

Cook and his associates have found two "neutral" solvents that are capable of dispersing gluten, namely 30% urea and 8–10% sodium salicylate. The latter has real possibilities and is decidedly preferable to the former. Its great advantage is that gluten dispersed in it apparently undergoes no observable hydrolysis or other change over long periods of time. As a disadvantage, it disperses some starch along with the protein, which is troublesome from the standpoint of isolating and studying the properties of pure, uncontaminated protein fractions.

Summarizing this fundamentally important phase of the problem then, in the light of present knowledge, we would conclude that there are available only two rational starting points for protein fractional precipitation studies that are apparently capable of yielding gluten fractions whose original properties have not been seriously altered. One involves dispersion in dilute acetic acid at ice temperature and the maintenance at that temperature during subsequent operations; and the other involves sodium salicylate as the dispersing agent. The former offers the advantage of permitting the complete removal of starch, while the latter has the convenient advantage of affording a reasonably permanent stability at room temperatures.

Neither time nor propriety will permit any detailed discussion of more than a few of the possibilities and operations involved in the fractional precipitations themselves. We have discovered and used a very large number of fractionation methods. Many of these, however, have involved the use of alcohol or acetone, and our recent observations relative to the consequences of the effects of alcohol or acetone on the lipoid-protein complex have caused us to realize the probability that such fractionations have distinct limitations. These methods will therefore be disregarded in this discussion.

When gluten dispersions, either in dilute acetic acid or in 8–10% sodium salicylate, are centrifuged at 30,000–40,000 r. p. m., there is at once removed—together with suspended starch—a fairly definite protein fraction which represents approximately 10–12% of the total. This is a fraction that apparently escapes complete dispersion, and which if not removed by the centrifuge would slowly settle out by gravity on standing. The composition and properties of this protein material do not correspond to those of any of the flour proteins that have been prepared by conventional methods and reported in the literature.

Having removed this protein, together with all suspended starch, the dispersed gluten can be fractionally precipitated from acetic acid dispersion by adding small, systematically varying amounts of salt. We have used both sodium chloride and sodium acetate. There are at least four variables influencing the amount of protein precipitated. These are, respectively, pH, quantity of salt, protein concentration and time. We have not found temperature to be a serious factor within fairly wide limits. The different fractions can be shown to vary in chemical composition (products of hydrolysis), which shows that we are dealing with mixtures. We are now confronted with the perplexing question of how to interpret and explain the meaning of these mixtures.

When these mixtures are found to vary systematically both in physical and in chemical properties, there are at least two possible interpretations. One is that there are innumerable components, as concluded by McCalla and Rose. The other is that there may be relatively few components, but that they are precipitated as mixtures containing progressively varying proportions of these few components. The possibility that the latter viewpoint could easily be correct is strongly indicated by some recent researches of the Dutch colloid chemist Bungenberg de Jong.

Fractionation from sodium salicylate gluten dispersions can be accomplished by the addition of salts, by diluting with water, by adding varying amounts of acid, and by additions of alcohol or acetone. Again, however, alcohol and acetone are prohibited if one wishes to keep the lipoid-protein fraction intact. Fractions precipitated from sodium salicylate dispersion present the same problem of interpretation as those from the acetic acid dispersions.

There still remains the question of whether the number of gluten components is large or small. Thus far, it would seem that fractional precipitation studies have not furnished trustworthy and conclusive evidence. It is quite possible that a more reliable answer to the question can be reached through fractional *solubility* studies than through fractional precipitation. By methods of fractional solubility, we are definitely able to avoid the formation of "adsorption" complexes or of those complexes that are described by Bungenberg de Jong, although we do have the matter of overlapping solubilities to contend with.

Without going into burdensome details, we conclude from some fractional solubility studies of this type, and from the data of other workers that, after all, the flour proteins are constituted of relatively few components, or component groups. Thus, in one of our experiments, we thoroughly extracted a flour with salt solution, and then repeatedly with distilled water. The combined water extractions, alone, removed more than 30% of the total flour protein. The successive fractions extracted with water were analysed individually, and *we found no differences in the chemical compositions of these fractions, respectively.* This indicates that there was at least more than 30% of the flour protein which was not made up of components that varied progressively and systematically in chemical compositions, and it definitely contradicts the evidence afforded by the fractional precipitation data of the type presented by McCalla and Rose.

In another experiment a single sample of flour was successively extracted with sodium salicylate solutions, starting with a dilute (0.5%) solution and then progressively increasing the concentration of salicylate by increments of one per cent, up to 10 per cent. Over this range nearly all of the protein is eventually dispersed. A comparison of the chemical compositions of the different fractions thus successively extracted revealed no trace of evidence of progressive and systematic variations in chemical properties, but again supported the idea that there are relatively few components or component

groups. Thus, as mentioned before, we have the two types of evidence, and it becomes largely a question of which, if either, of the two types is the more trustworthy.

Our opinions and our beliefs as to the actual nature and character of the wheat flour proteins have undergone repeated changes and modifications during the period over which we have studied them. In view of the complexities involved, however, we offer no apologies or excuses. We can merely summarize our *present* viewpoint, which is as follows: There is a considerable portion of flour protein that does not enter into the formation of gluten, but it is not yet possible to distinguish a sharp and definite boundary-line between the gluten and non-gluten portions. With reference to gluten, itself, we recognize a definite fraction consisting of approximately 10–12% that is extremely resistant to dispersion. We recognize the "gliadin" fraction, which accounts for 50% or more of the gluten, and which may or may not be a mixture of components having very similar properties. The remaining portion we regard as doubtful, but until proven otherwise we prefer to look upon it as a mixture of a "glutenin" and a lipoid-protein complex. We consider it possible that the fraction which we once designated as "mesonin" is in reality the protein fraction that is liberated upon the disruption of the lipoid-protein complex by alcohol. This, however, is merely a speculative guess, based upon meager circumstantial evidence.

At present we feel that a thorough study and understanding of the nature and properties of the lipoid-protein complex will contribute greatly to the solution of several flour problems that are of the greatest fundamental interest and importance. Sell, Olsen and Kremers have identified lecitho-protein as the emulsifying and stabilizing ingredient of egg yolk in making mayonnaise, and it may not be too far-fetched to imagine an analogous situation in bread dough.

The lipoid portion of the complex is seemingly that portion of the total flour lipoid extractable with ether only after pre-treatment with alcohol or with acetone. The complex is apparently destroyed and largely removed when any preparation containing it is dried with alcohol and ether, which is, of course, the time-honored method for drying protein preparations. This is very discouraging to our prospects of finding any means to isolate the complex in pure dry form. We have, however, separated—but not dried—a fraction representing 12% of the entire gluten that yielded 37% of its weight as lipoid. In another instance a certain gluten fraction representing about 5%

of the total had a lipoid content of 44%. These are very considerable amounts. Our studies thus far indicate that the entire crude gluten may contain from 5 to 10% of this lipoid, which is in fairly good agreement with the findings of Dill.

I conclude this discussion in the hope that I have at least been able to indicate some of the reasons why progress in the various efforts to gain an insight into the fundamental nature and properties of the flour proteins has been so painfully slow and uncertain, and I have only touched upon a few of the major difficulties. It is peculiarly a problem in which any new discovery is likely to invalidate much that has gore before and to necessitate an entire re-examination of the whole situation. It is certain, however, that no complete understanding of flour properties and problems can ever be realized until the fundamental nature of the flour proteins has been adequately disclosed.